Pertanika Journal of

TROPICAL AGRICULTURAL SCIENCE

JTAS

VOL. 37 (2) MAY 2014

PERTANIKA JOURNALS

A scientific journal published by Universiti Putra Malaysia Press

Journal of Tropical Agricultural Science

About the Journal

Pertanika is an international peer-reviewed journal devoted to the publication of original papers, and it serves as a forum for practical approaches to improving quality in issues pertaining to tropical agriculture and its related fields. Pertanika Journal of Tropical Agricultural Science which began publication in 1978 is a leading agricultural journal in Malaysia. After 29 years as a multidisciplinary journal, the revamped Pertanika Journal of Tropical Agricultural Science (JTAS) is now focusing on tropical agricultural research. Other Pertanika series include Pertanika Journal of Science and Technology (JST) and Pertanika Journal of Social Sciences and Humanities (JSSH).

JTAS is published in **English** and it is open to authors around the world regardless of the nationality. It is currently published four times a year, i.e. in **February**, **May**, **August** and **November**.

Goal of *Pertanika*

Our goal is to bring the highest quality research to the widest possible audience.

Quality

We aim for excellence, sustained by a responsible and professional approach to journal publishing. Submissions are guaranteed to receive a decision within 12 weeks. The elapsed time from submission to publication for the articles averages 5-6 months.

Indexing of *Pertanika*

Pertanika is now over 33 years old; this accumulated knowledge has resulted in Pertanika JTAS being indexed in SCOPUS (Elsevier), Thomson (ISI) Web of Knowledge [BIOSIS & CAB Abstracts], EBSCO, DOAJ, AGRICOLA, ISC, MyAIS and Rubriq.

Future vision

We are continuously improving access to our journal archives, content, and research services. We have the drive to realise exciting new horizons that will benefit not only the academic community, but society itself.

We also have views on the future of our journals. The emergence of the online medium as the predominant vehicle for the 'consumption' and distribution of much academic research will be the ultimate instrument in the dissemination of research news to our scientists and readers.

Aims and Scope

Pertanika Journal of Tropical Agricultural Science aims to provide a forum for high quality research related to tropical agricultural research. Areas relevant to the scope of the journal include: *agricultural biotechnology, biochemistry, biology, ecology, fisheries, forestry, food sciences, genetics, microbiology, pathology and management, physiology, plant and animal sciences, production of plants and animals of economic importance,* and *veterinary medicine*.

Editorial Statement

Pertanika is the official journal of Universiti Putra Malaysia. The abbreviation for Pertanika Journal of Tropical Agricultural Science is *Pertanika J. Trop. Agric. Sci.*

JTAS

Journal of Tropical Agricultural Science

AN INTERNATIONAL PEER-REVIEWED JOURNAL

EDITORIAL OFFICE

JOURNAL DIVISION
Office of the Deputy Vice Chancellor (R&I)
1st Floor, IDEA Tower II
UPM-MTDC Technology Centre
Universiti Putra Malaysia
43400 Serdang, Selangor Malaysia.
Gen Enq.: +603 8947 1622 | 1619 | 1616
E-mail: executive_editor.pertanika@upm.my
URL: www.journals-jd.upm.edu.my

PUBLISHER

Kamariah Mohd Saidin
UPM Press
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor, Malaysia.
Tel: +603 8946 8855, 8946 8854
Fax: +603 8941 6172
E-mail: penerbit@putra.upm.edu.my
URL: http://penerbit.upm.edu.my

ABSTRACTING/INDEXING

Pertanika is now over 35 years old; this accumulated knowledge has resulted the journals being indexed in SCOPUS (Elsevier), Thomson (ISI) Web of Knowledge [BIOSIS & CAB Abstracts], EBSCO, DOAJ, Google Scholar, AGRICOLA, ISC, Citefactor, Rubriq and MyAIS.

Pertanika Journal of Tropical Agricultural Science
Vol. 37 (2) May 2014

Contents

Foreword

Nayan Deep S. Kanwal i

Review Article

Senescence and Postharvest Studies of Cut Flowers: A Critical Review 159
Pooja Rani and Narender Singh

Regular Articles

The Enzyme Activities of Pancreas and Small Intestinal Contents in the Malaysian Village Chicken and Broiler Strains 203
Khalid K. Kadhim, Md Zuki Abu Bakar, Noordin Mohamed Mustapha, Mohd Amin Babjee and Mohd Zamri Saad

The Responses by Gut-Associated and Bronchus-Associated Lymphoid Tissues of Buffalo Calves Following Oral Exposure to *Pasteurella multocida* B:2 215
M. S. Abu Bakar, Mohd Zamri Saad, S. Jasni and Zuki Abu Bakar

Increasing Rice Production Using Different Lime Sources on an Acid Sulphate Soil in Merbok, Malaysia 223
Elisa Azura Azman, Shamshuddin Jusop, Che Fauziah Ishak and Roslan Ismail

Cattle Grazing Effect on *Mimosa pudica* L. in Tropical Pasture System 249
Majid Ajorlo, Ramdzani Abdullah, Ridzwan Abdul Halim and Mahboubeh Ebrahimian

Stored Carbon in Dominant Seaweeds of Indian Sundarbans 263
Mitra, A., Zaman, S., Pramanick, P., Bhattacharyya, S. B. and Raha, A. K.

Mixed Viral Infection and Growth Stage on Chilli (*Capsicum annuum* L.) Production 275
Nurhayati Damiri

Determination of *Pediobius* sp. (Hymenoptera: Eulophidae), A New Species Record of Endoparasitoid Associate with Beet Armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidea) from Malaysia using DNA Barcode 285
Ghazali, S. Z., Md-Zain, B. M. and Yaakop, S.

Foreword

Welcome to the **Second Issue 2014** of the Journal of Tropical Agricultural Science (JTAS)!

JTAS is an open-access journal for the Tropical Agricultural Science published by Universiti Putra Malaysia Press. It is independently owned and managed by the university and run on a non-profit basis for the benefit of the world-wide science community.

This issue contains **eight articles,** out of which **one** is a review article and **seven** are regular research papers. The authors of these articles are from **Malaysia, India, Iran** and **Indonesia**.

The review paper discusses senescence and postharvest studies of cut flowers (*Pooja Rani* and *Narender Singh*). Postharvest events in floricultural crops reflecting petal senescence are reviewed in this paper. This review also pays attention to issues related to carbohydrate metabolism and change in anthocyanin pigmentation during postharvest life.

The seven research papers cover a wide range of topics. In the first research paper, researchers from Universiti Putra Malaysia examine the enzyme activities of the contents of the pancreas and small intestines in the Malaysian village chicken and broiler strains (*Khalid K. Kadhim, Md Zuki Abu Bakar, Noordin Mohamed Mustapha, Mohd Amin Babjee* and *Mohd Zamri Saad*). The next research paper discusses the responses by gut-associated and bronchus-associated lymphoid tissues of buffalo calves following oral exposure to *Pasteurellamultocida* B:2 (*M. S. Abu Bakar, Mohd Zamri Saad, S. Jasni* and *Zuki Abu Bakar*). The following research paper, which was done locally at Merbok, Malaysia,shows that the use of different lime sources on an acid sulphate soil will significantly increase rice production (*Elisa Azura Azman, Shamshuddin Jusop, Che Fauziah Ishak* and *Roslan Ismail*).

The next research paper discusses the effect of cattle grazing on *Mimosa pudica* L. in the tropical pasture system (*Majid Ajorlo, Ramdzani Abdullah, Ridzwan Abdul Halim* and *Mahboubeh Ebrahimian*)while the following research paper discusses on stored carbon in the dominant seaweeds (*Enteromorpha intestinalis, Ulvalactuca* and *Catenellarepens*) of the Indian Sundarbans (*Mitra, A., Zaman, S., Pramanick, P., Bhattacharyya, S. B.* and *Raha, A. K*).

Researchers from the Sriwijaya University, Indonesia have studied the effect of mixed viral infection and growth stage on chilli (*Capsicum annuum* L.) production (*Nurhayati Damiri*). The last research paper is from a group of researchers from Universiti Kebangsaan Malaysia who successfully report on a new species of endoparasitoid associate with beet armyworm, *Spodopteraexigua* (Lepidoptera: Noctuidea) from Malaysia using DNA barcode (*Ghazali, S. Z., Md-Zain, B. M.* and *Yaakop, S.*).

I anticipate that you will find the evidence presented in this issue to be intriguing, thought-provoking and useful in reaching new milestones in your own research. Please recommend the journal to your colleagues and students to make this endeavour meaningful.

I would also like to express my gratitude to all the contributors, namely, the authors, reviewers and editors who have made this issue possible. Last but not least, the editorial assistance of the journal division staff is fully appreciated.

JTAS is currently accepting manuscripts for upcoming issues based on original qualitative or quantitative research that opens new areas of inquiry and investigation.

Chief Executive Editor
Nayan Deep S. KANWAL, FRSA, ABIM, AMIS, Ph.D.
nayan@upm.my

Pertanika J. Trop. Agric. Sci. 37 (2): 159 - 201 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Review Paper

Senescence and Postharvest Studies of Cut Flowers: A Critical Review

Pooja Rani and Narender Singh*

Department of Botany, Kurukshetra University, Kurukshetra, 136119 Haryana, India

ABSTRACT

Flower senescence is the terminal phase of developmental processes that leads to the end of its life span. Since a number of developing countries are attracted to this global fresh flower trade for commercial purpose, this phenomenon is major obstacle for all the floricultural industries. Therefore, research related to postharvest changes was carried out to mitigate this problem. The post-harvest events in floricultural crops reflecting petal senescence are being reviewed in this paper, whereby various physiological and biochemical studies having data regarding lipid peroxidation, loss of membrane integrity and protein degradation central to petal senescence are included. Ultrastructural changes involving change in various cell organelles viz. rupturing of vacuole, tonoplast membrane invagination, chloroplast degradation in mesophyll cells, as well as change in mitochondria ultrastructure have also been recited. This review also pays attention to the issues related to carbohydrate metabolism and change in anthocyanin pigmentation during postharvest life. Various enzymatic activities, supporting petal senescence and current status of post-harvest technology applied to cut flowers to enhance their vase life especially by using preservatives in the form of energy source like sucrose and other sugars, biocides, mineral ions, growth regulators or various metabolic inhibitors, providing practice solution to global cut flower market, are cited.

Keywords: Cut flowers, postharvest, ultrastructural changes, senescence, biochemical changes, growth regulators

ARTICLE INFO

Article history:
Received: 4 April 2013
Accepted: 10 September 2013

E-mail addresses:
poojabishan@gmail.com (Pooja Rani),
nsheorankuk@yahoo.com (Narender Singh)
* Corresponding author

INTRODUCTION

Owing to a steady increase in demand of flowers, floriculture has become one of the important commercial trades in agriculture. Floriculture is now seen as a high growth

ISSN: 1511-3701

industry from export angle and is therefore a lucrative business. The production and export of floriculture crops from developing countries provide trade and currency. Investments in floriculture in developing countries can serve to decrease many social ills including poverty, terrorism, and illegal drug trafficking. Global exports over the last few years have grown by more than 10% annually, and at this growth rate, the world exports are expected to reach US$ 25 billion by 2012. In order to meet this growing and changing demand, production has continued to move from countries that have traditionally been consumers and growers, such as the Netherlands, to other relatively new producing countries such as Israel, Colombia, Ecuador, Kenya, and Ethiopia. The research provides valuable knowledge about the execution of senescence in plants or plant parts like leaf, petal or sepal and how senescence is influenced by biotic and abiotic factors like environmental stresses and what physiological and biochemical changes occur during this process. This information will be used to increase the shelf life of flowering plants, which will reduce postproduction shrink and increase the profitability of floriculture producers. Therefore, since from past few years, postharvest physiology of flower has been gaining much attention to study this phenomenon of senescence and various techniques are being designed to slow down this process.

Flowers play a vital role in angiosperm reproduction; they are often pigmented and or perfumed to attract pollinators. However, despite its irreplaceable ecological role, the flowers are energetically expensive to maintain beyond their useful life, and therefore have a limited life-span that is usually taken away after pollination; causing senescence syndrome. Senescence of flower is a complex process, so often researchers mainly concentrate on changes occurring during petal senescence. Petals provide an excellent model system for the study of fundamental aspects of senescence (Rogers, 2006; Desai *et al*., 2012). Senescence is a highly regulated final event of flower development that bears hallmarks of programmed cell death (PCD), resulting in colour changes, petal wilting, abscission of whole flower and flower parts with various physiological, biochemical and ultrastructural changes (Voleti *et al*., 2000; Wagstaff *et al*., 2003; Jones *et al*., 2005; Tripathi & Tuteja, 2007; Seo *et al*., 2009; Ichimura, 2010; Shahri, 2011). Recent studies evidenced that flower senescence includes controlled disassembly of cells of corolla probably by a mechanism homologous with apoptosis, vacuolar and necrotic PCD (Van Doorn, 2011), and transport of nutrient to other parts of inflorescence. The most important barriers in the marketing and commercialization of many cut flowers are their short vase life and their inability to withstand stresses during storage or transit (Halevy & Mayak, 1981; Nowak & Rudnicki, 1990; Zamani *et al*., 2011). A great deal of research dealing with best post-harvest care of cut flowers has been carried out in recent years but our understanding of cut flower physiology is

still quite rudimentary, despite development of techniques that have enabled us to maintain good cut flower quality, longer than ever before. Petals are the main floral organs which primarily determine the commercial longevity of flowers and as a consequence, it becomes necessary to study the physiological, biochemical and genetic processes that occur during petal senescence (Chakrabarty *et al.,* 2009) and how it can be slowed down through designing inexpensive postharvest technologies that ultimately extend the postharvest life of cut flowers (Wani *et al*., 2012). This review is expected to give an update of literature on postharvest behaviour of cut flowers and some of the recent technologies contributing to their postharvest life which is major priority for growth of such a global floriculture industry. It also comprehends metabolic changes regarding protein degradation, lipid peroxidation, alteration in sugar levels in phloem exudates, activity of various enzymes and colour change central to petal senescence during post-harvest life of cut flowers to know exact the mechanism of senescence during post-harvest life and also entails the use of various preservatives or holding solutions like sugars, biocides, mineral ions, growth hormones and metabolic inhibitors, etc. to mitigate the problem of short post-harvest life and how they serve to retard the petal senescence. There are some other practices are not discussed here.

POST-HARVEST CHANGES ASSOCIATED WITH SENESCENCE

Ultrastructural Changes

There is a three-stage theory of senescence in case of flowers like those in leaf. First is the initiation of senescence followed by degradation and disassembly which lead to third stage of death (Yoshida, 2003), which is due to decline in rate of anabolic processes and increase in rate of certain catabolic processes (Galston & Davies, 1970). Characteristics of the last phase involve ultrastructural disorganization of tissues or cells and increased fluid filled extra spaces which lead to halted down of all metabolic activities in all tissues or organs of plant. But, some organelles are still slightly visible (Smith *et al*., 1992). Delicacy of petal cells and their rapid collapse during senescence are a challenge to study ultrastructural changes during senescence and showed dramatic changes in ultrastructure (Van Doorn *et al*., 2003). Ultrastructural events during senescence include increase in vacuolar size, loss of organelles, eventual collapse of tonoplast (Van Doorn & Woltering, 2004) and nuclear fragmentation (Yao *et al*., 2004; Yamada *et al*., 2006; Battelli *et al*., 2011). Wiemken *et al*. (1976) used *Iris* as a model system to study ultrastructural, biochemical and molecular changes during senescence. One of the earliest changes in ultrastructure of *Iris* petals is closure of plasmodesmata. Plasmodesmata if open allow transfer of small molecules like sugars, hormones and RNA molecules between adjacent cells. If plasmodesmata are closed, transport

gets halted. Ultrastructural work in *Iris*, *Sandersonia***,** senescing corolla of *Lycoris longituba* Y.C. Hsu & G.J. Fan and *Lilium longiflorum* also showed complete degradation of wall of mesophyll cells prior to visible senescence due to closure of plasmodesmata whereas epidermal cells remain intact (Wagstaff *et al*., 2003; Van Doorn *et al*., 2003, O'Donoghue *et al*., 2005; Lei *et al*., 2009; Battelli *et al*., 2011). Other ultrastructural events involve invagination in tonoplast and presence of numerous vesicles in vacuole which is main site of organelle degradation (Matile & Winkenbach, 1971; Smith *et al*., 1992). As senescence proceeds, all cytoplasmic content get lost in carnation (Smith *et al*., 1992) and *Iris* (Van Doorn *et al*., 2003). The increases in the number of small vacuoles and size of vacuole have also been evidenced in carnation (Smith *et al*., 1992), *Iris* (Van Doorn *et al*., 2003) and *Hemerocallis* (Stead & Van Doorn, 1994). Meanwhile, disappearance of free ribosomes and clusters attached to endoplasmic reticulum during maturation and senescence followed by reduction in the number of mitochondria, golgi bodies followed by other organelles, was also recited (Butler & Simon, 1971; Smith *et al*., 1992; Van Doorn *et al*., 2003). Most noticeable changes during development and senescence take place in plastids show invaginations in plastid membrane. Changes in the structure of tissue containing chromoplasts have also been observed in cucumber petals (Smith & Butler, 1971) and amyloplasts in *Lycoris longituba* Y.C. Hsu & G.J. Fan (Lei *et al*., 2009). With reference to the chloroplast ultrastructure, a higher level of thylakoid disorganization (especially of granal membranes) is observed during senescence (Spundova *et al*., 2003). The loss of non-cellulosic natural sugar and the increase in soluble pectin, uronic acid and cellulose (De Vetten & Huber, 1990; Smith *et al*., 1992) lead to alternation in cell wall and initiation and increase in the loss of membrane integrity resulting in phase changes due to decrease in membrane fluidity and increased permeability (Thompson *et al*., 1982; Knowles *et al*., 2001). The event related to the loss of RNA and DNA present in nucleus, mitochondria, chloroplast and plastids is also associated with senescence (Rubinstein, 2000; Aleksandrushkina *et al*., 2008; Lei *et al*., 2009) before or after vacuolar collapse. More detailed analysis should be carried out at cell and organelle levels to determine exactly what is happening inside the petal during senescence and how we can retard this syndrome of senescence.

Colour Fading and Change in Pigmentation

Chlorophyll is the most prominent photosynthetic pigment in higher plants and the decreasing trend in photosynthetic rate and pigment level is generally due to the involvement of oxygen radicals and singlet oxygen (Prochazkova *et al*., 2001; Dertinger *et al*., 2003). Chlorophyll breakdown becomes a mandatory phenomenon for the remobilization of nitrogen from chlorophyll-binding proteins to proceed during senescence (Hortensteiner, 2006). Louda *et al*. (2002) and Spundova *et al*.

(2003) found that most chlorophyll species were broken down during senescence. However, considerable delay in degradation of chlorophyll compared with control and copper sulphate was found by keeping the flower in aluminium sulphate and ethanol by slowing down the rate of transpiration and deterioration (Hajizadeh *et al*., 2012).

Colour fading and discoloration are major reasons for termination of vase life in many cut flowers and important factors in determining display quality of cut flowers. Major three types of pigments contributing to the colour of flowers are anthocyanins, carotenoids, and betalains. Anthocyanins are the largest and most diverse group of plant pigments derived from the phenylpropanoid pathway, ranging in colour from red to violet and blue. Anthocyanins give red colour under low pH and blue colour under high and neutral pH, reflecting a phenomenon termed as 'blueing' where a shift from red to blue occurs with ageing (Wills *et al*., 1998; Avila-Rostant *et al*., 2010). Ultimate flower colour of a species is determined not only by the pigment present but also by various other factors like pH (Harborne, 1988; Yoshida *et al*., 2003; Katsumoto *et al*., 2007), temperature (Shvarts *et al*., 1997; Dela *et al*., 2003), light (Weiss, 2000; Meng & Wang, 2004; Irani & Grotewold, 2005), and mineral ions (Shoji *et al*., 2007). Griesbach (2005) observed that although flavonols and an appropriate pH are important in obtaining blue orchids, the more important of the two factors was vacuolar pH. In morning glory, the colours of flower vary from reddish purple in buds to blue in flowers with an increase in vacuolar pH from 6.6 to 7.7, a change believed to be driven by a $Na^+(K^+)/H^+$ exchanger (Yamaguchi *et al*., 2001; Yoshida *et al*., 2005). Collette *et al*. (2004) and Elibox and Umaharan (2008) investigated the relationship between epidermal vacuolar pH and a number of plant factors in anthurium with the intention of creating colours in the blue range by characterizing the genetics and biochemistry of the anthurium flavonoid biosynthetic pathway. In flowers, degradation of anthocyanins during senescence is possibly related to oxidative process. A significant increase in antioxidant activity is correlated with the rate of anthocyanin degradation (Mazza, 2007). Reducing agents such as glutathione can inhibit the degradation of anthocyanins (Vaknin *et al*., 2005). In morning glory (*Ipomoea tricolor*) petals, the vacuolar pH is relatively low when the flower bud opens, resulting in a red colour but upon further maturation, the vacuolar pH increases and the petals acquire a strong blue colour, but in mutants, colour change does not occur and remains purple (Yoshida *et al*., 1995).

Rose flowers produced under cooler environment have higher anthocyanin content especially during summer (Plaut *et al*., 1979). High temperature applied at different stages of flower development reduces anthocyanin content in petals (Dela *et al*., 2003). High temperature and low light conditions have also been reported to reduce pigment content in petals. This is due to the breakdown and down regulation of genes encoding enzyme involved in biosynthesis of anthocyanins (Gonzalez, 2009).

Low light intensity plants develop pale flowers with a low level of anthocyanins (Biran & Halevy, 1974; Griesbach, 1992). In *Eustoma grandiflorum* Shinn., low light conditions result in reduced anthocyanin content in petals of developing florets both in cut flowers and potted plants measured by the expression pattern of six genes encoding enzymes for anthocyanin biosynthetic pathway in developing petals concluded that light intensity regulates a master transcription factor common for all these anthocyanin biosynthesis genes (Meir *et al*., 2009). Low light intensity affects petal pigmentation through reduced photosynthesis in the leaves or stems, which in turn reduces the soluble sugar content of petals and leads to a repression of the genes that encode enzymes of anthocyanin biosynthetic pathway in *Eustoma grandiflorum* (Kawabata *et al*., 1995). Therefore, increased sucrose concentration has been found to enhance petal growth and pigmentation in detached flowers of *Eustoma grandiflorum* and rose (Kuiper *et al*., 1991; Sankhla *et al.*, 2005). The influence of sucrose and light intensity on lightness, chroma and petal colour of flower has also been reported through change in anthocyanin pigmentation in lilianthus cultivars (Uddin *et al*., 2001). The induction of anthocyanin synthesis and anthocyanin biosynthetic gene expression in detached petunia *(Petunia hybrida)* corollas by gibberellic acid (GA_3) requires sucrose for activation of anthocyanin biosynthetic gene (Moalum-Beno *et al*., 1997).

Carotenoids like anthocyanins are also widely distributed in angiosperms whereas betalains are only found in some plants in the order Caryophyllales in some higher fungi such as *Amanita muscaria*. Betalains have functions analogous to those of anthocyanins as pigments. The majority of carotenoids in the petals of sandersonia (*Sandersonia aurantiaca*) are β, β-carotenoids such as β-cryptoxanthin, zeaxanthin and β-carotene (Nielsen *et al.*, 2003). On the other hand, more than 90% of the carotenoids in the petals of (*Tagetes* sp.) marigold (Moehs *et al.*, 2001) and chrysanthemum (Kishimoto *et al.*, 2004) are lutein and/ or lutein derivatives. There are many carotenoids whose biosynthesis has not been characterized.

Taken together, it is concluded that anthocyanins, carotenoids and betalains constitute the majority of the flower pigments which can be affected by various factors like pH, light, temperature, etc. Some preservatives like sugar and growth hormones like GA_3 might prove to be best in delaying degradation of pigments like anthocyanins. At present, biosynthetic pathway of these pigments and their regulation are well known. However, the mechanisms of transport and sequestration of these pigments inside the vacuole which petals show variable colour is not known. Molecular approach may prove to be beneficial to know the mechanism of transport and their sequestration.

Lipid Peroxidation and Loss of Membrane Integrity

Lipid peroxidation generates a range of reactive oxygen species, including singlet oxygen ($^1O_2$), the alkoxy radical ($RO^{\bullet}$),

hydroxyl radical ($OH^{\bullet}$) and hydroperoxyl radical ($HO_2^{\bullet-}$), and the peroxy radical ($ROO^{\bullet}$) which amplify the lipid peroxidation with further degradation of released fatty acids affecting membrane permeability (Van Doorn & Woltering, 2008; Rogers, 2012). All these can oxidize a range of macromolecules with varying specificity, although the hydroxyl radical is the most reactive and least specific (Dat *et al*., 2000). Bieleski and Reid (1992) found evidence for rapid cessation of overall phospholipids synthesis during petal senescence in *Hemerocallis*. All the enzymes required for phospholipid degradation are apparently present in membranes, even in those of young cells (Brown *et al*., 1990). Microsomal membranes from the petals of senescing carnation (*Dianthus caryophyllus* L.) flowers contain phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. These phospholipid classes decline essentially together during natural senescence of the flower (Brown *et al*., 1991; Chakrabarty *et al*., 2009; Lei *et al*., 2009). The decrease in levels of various lipids is associated with plant senescence. During petunia (*Petunia hybrida* Vilm.) flower senescence, there is a senescence-related increase in the content of diacylglycerol, one of the products of phospholipids metabolism in plasma membranes (Borochov *et al*., 1997).

The main phospholipid degrading enzymes, including (i) Phospholipase D (ii) Phospholipase C (iii) Lipolytic acyl hydrolase and (iv) Lipooxygenase, which degrade fatty acids were reported to be upregulated during petal senescence. Degradation of free fatty acids mainly occurs by *β*-oxidation (Pistelli *et al*., 1992), end product of which is fed into glyoxylate cycle and converted to sugars in cytosol (Chen *et al*., 2000; Cornah *et al*., 2004) or used in synthesis of amino acids. Acyl-CoA oxidase isozymes catalyse the first step in peroxisomal fatty acid *β*-oxidation. The transcript abundance of a gene encoding an acyl-CoA synthase increased during senescence making plasma membrane permeable prior to vacuolar collapse due to phospholipid degradation inside membrane (Hopkins *et al*., 2007). Freeze-fracture electron microscopy of senescing carnation petals indicated the presence of gel phase lipid in plasma membrane, ER and tonoplast showing senescence syndrome (Hopkins *et al*., 2007). The ratio of saturated/unsaturated fatty acids was also found to increase due to extensive degradation of phospholipids and galactolipids during flower senescence (Leverentz *et al*., 2002).

Cellular membranes are selective, dynamic barriers, structural integrity of which is necessary for critical membrane functions such as maintaining the cell's osmotic balance in petals. The rupture of cellular membrane is likely to be deleterious to plant function since regulation of metabolites and signal exchange between neighbouring cells will also be lost. Membrane damage may occur early or later in the process of senescence. The accumulation of peroxidized lipids and products derived from them in senescing membranes appears to cause extensive destabilization of the membrane bilayer

structure and loss of membrane function. Thompson (1988) also observed a strong correlation between membrane leakiness and phospholipid breakdown in senescent flowers, rendering the membrane more susceptible to lipid degrading enzymes such as lipooxygenase that leads to broken down of cell membrane (Hildebrand & Hymowitz, 1982; Fobel *et al*., 1987; Lei *et al*., 2009). Electrical conductivity of the petal diffusates reached maximum at full bloom, with significantly higher values in *Rosa damascene* (Sood *et al*., 2006). Membrane permeability of sepal tissues estimated as electrical conductivity of ion leachates was also observed to increase as the development proceeded through various stages in *Ranunculus asiaticus* L. and *Consolida ajacis* L. cv. 'Violet Blue' (Shahri & Tahir, 2011a, b). Fukuchi-Mizutani *et al*. (2000) noticed increased activity of lipooxygenase (LOX) with senescence of cut flowers stimulating deterioration of membrane. Deterioration of cellular membranes causes increased membrane permeability, loss of ionic gradients and decreased function of key membrane proteins (e.g., ion pumps). Nevertheless, it is pertinent to note that loss of membrane function in *Alstroemeria* has been shown to occur without increased activity of lipooxygenase suggesting that the loss of membrane integrity can be achieved in a number of ways (Leverentz *et al*., 2000). Membrane breakdown in lupin (Hernandez-Jimenez *et al*., 2002), carnation (Bartoli *et al*., 1995), day lily (Rubinstein, 2000), rose (Sood *et al*., 2006) and gladiolus (Hossain *et al*., 2006) is evidenced by positive role of lipooxygenase in promoting senescence. With loss of the integrity of cellular membrane structure in cells undergoing senescence, hydrolytic enzymes being normally compartmentalized in these cells are released and could cause massive breakdown of various cellular components. There are various changes related to membrane in plant tissue. In some cases, lipid pentadienyl, peroxyl free radicals may also be generated by LOX action (Roy *et al*., 1994). The result obtained by Brown *et al*. (1987) evidenced the microsomal membrane from carnation produced increased quantity of O_2^- during senescence. According to the authors, O_2^- is produced by membrane bound oxidase. Lipid peroxidation not only threatens membrane proteins and functioning and integrity of membrane but also produces a variety of toxic aldehyde and ketones (Wilhelmova *et al*., 2006). A marked deterioration of the plasma membrane and loss of water are associated with lipid peroxidation with the senescence of chrysanthemum and *Hemerocallis* petals (Bartoli *et al*., 1995; Bartoli *et al*., 1997; Chakrabarty *et al.,* 2009). Therefore, membrane degradation may be a central step in the process of senescence that leads to mass lipid degradation during senescence and collapse of the tonoplast which results in executing the death sentence. The main controversy is about the main site of phospholipid degradation and to what extent this phospholipid degradation takes place inside the vacuoles and autophagosomes and to what extent inside the membrane.

Loss of Cellular Protein

Proteins are the key molecules that play important roles in various structural and functional aspects of plants. Senescence in tepals, stamens and carpels results in an increase in total protease activity and a decrease in total protein content. In many species of plants, protein degradation and remobilization are mediated through protein ubiquitination and the action of specific proteases (Wagstaff *et al*., 2002; Pak & Van Doorn, 2005; Batelli *et al*., 2011) transferring various amino acids to phloem. The soluble proteins registered a consistent decrease with the simultaneous increase in specific protease activity and α-amino acid content during different stages of flower development and senescence in *Ranunculus asiaticus* L. and *Consolida ajacis* cv. Violet Blue (Shahri & Tahir, 2011a, b). In many species of other flowers like *Petunia* and rose, a drastic decrease was found in protein level prior to visible senescence symptoms (Jones *et al*., 2005; Sood *et al*., 2006). An increase in amino acid content in phloem exudates from flower opening to petal wilting in *Ipomoea, Hemerocallis* and *Sandersonia* petals was observed due to protein degradation. Asparagine, lysine, glutamine and hydroxyproline were main transport amino acids (Wiemken *et al*., 1976; Bieleski, 1995; Eason *et al*., 2002). Several genes like DEAD/DEAH domain helicase related to protein synthesis are differentially expressed during petal senescence, both in daffodil (Hunter *et al*., 2002) and in *Alstroemeria* (Breeze *et al*., 2004). These genes are homology to the eIF-4A. Actinomycin D, an inhibitor of transcription, if given 4 h prior to opening, suppressed the onset of visible senescence, which occurred at about 9 h after flower opening by downregulation of senescence associated genes in petals of morning glory (Yamada *et al*., 2007). SDS-PAGE of protein extract from sepal tissues of *Helleborus orientalis* suggested a decrease in the expression of high molecular weight proteins and an increase in low molecular weight proteins during flower development and senescence. At present, it is not known whether the polypeptides that increased during senescence play an important role in the senescence of *Helleborus orientalis* flowers but these polypeptides may be linked to longevity (Shahri *et al*., 2011).

In proteasomes dependent degradation, proteasome system involved in degradation of specific proteins was apparently up-regulated during petal senescence in (*Hemerocallis*) day lily (Müller *et al*., 2004) and daffodil (Hunter *et al*., 2002). In carnation, the abundance of mRNA increased three genes encoding subunits of the 19S regulatory particle, one of two large complexes of the 26S proteasome (Hoeberichts *et al*., 2007). A significant delay in the time to visible senescence was observed through feeding the isolated *Iris* petals with Z-Leu-Leu-Nva-H, an inhibitor of proteasome activity (Pak & Van Doorn, 2005). Ubiquitinated proteins involved in the degradation of many petal proteins during floral development and senescence increased in intensity as the flowers senesced. Several monomers of

ubiquitin (a 76 amino acid polypeptide) become attached to protein targeted for degradation in the proteosomes having involvement of three enzymes referred to as E1, E2 and E3. Silencing of RING domain of E3 protein delayed visible senescence symptoms in *Petunia* (Xu *et al*., 2008). Expression of a homologous gene encoding a RING zinc finger ankyrin repeat protein (MjXB3), a putative E3 ubiquitin ligase, in petals of senescing four o'clock (*Mirabilis jalapa*) flowers highly increased during the onset of visible senescence like that in *Petunia* (Xu *et al.,* 2007). Silencing of this gene also resulted in extension of flower life.

Proteasome-independent protease activity increases prior to visible senescence (Stephenson & Rubinstein, 1998; Pak & Van Doorn, 2005). The proteases are often divided into exo- and endoproteases, indicating position of the target protein from where the cleavage takes place. Endoproteases include cystiene-, serine-, aspartic-, and metalloproteases, named after the amino acid residues or the metals that are required for cleavage reaction. In gladiolus, of the total protease activity, serine proteases account for about 67–70% while cysteine proteases account for only 23–25% (Azeez *et al.*, 2007). E-64 and antipain, these are specific inhibitors used for assessing the activities of proteases, both affected cysteine proteases. Total protease activity was reduced in petals of *Hemerocallis* (Stephenson and Rubinstein, 1998) and *Gladiolus* (Arora & Singh, 2004) and in *Petunia* (Jones *et al*., 2005) by using E-64 *in vitro*. Antipain reduced protease activity of *Sandersonia* petals by 30% (Eason *et al*., 2002). Feeding *Iris* petals with a membrane permeable form of E-64 also indicated that about half of the peak protease activity was due to cysteine proteases (Pak & Van Doorn, 2005). In carnation petals, a gene encoding a cystiene inhibitor, abundant at the time of flower opening, became gradually down regulated. Its mRNA had disappeared by the time of the increase in cysteine protease expression and petal wilting (Sugawara *et al.,* 2002). The cysteine protease inhibitor 2, 2-dipyridyl delayed the time to wilting in *Sandersonia* petals and prevented the senescence-associated rise in endoproteases activity (Eason *et al*., 2002). Thus, petal senescence is accompanied by bulk non-proteasomal protein degradation, mainly in vacuoles. This process of protein degradation shows accumulation of considerable amount of amino acids during senescence. Many genes which encode protein for the proteases have also been discovered to retard this process. To some extent, this molecular approach has been proved to be involved in regulation and inhibition of proteases. Further work is also needed to prevent degradation of protein at gene level or using some cultural practices like using preservatives or inhibitors to slow down the activity of proteases.

Enzymatic Activities

An unavoidable consequence of aerobic metabolism is production of reactive oxygen species (ROS) which may be beneficial or deleterious depending upon the concentration of ROS. A high concentration

leads to damage of biomolecules and low concentration act as second messenger that mediate several responses in plants. When concentration of ROS becomes high, antioxidant system comprising of enzymatic (ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and guaiacol peroxidase (GPX) and nonenzymatic components (Ascorbate, glutathione, tocopherols, carotenoids and phenolic compounds) is there in plants to scavenge these ROS. Here, the main emphasis is given on enzymatic components like ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and guaiacol peroxidase (GPX), etc., which show antioxidant behaviour with progressive senescence. Peroxidases are one of the important enzymes found in plant material which bleaches chlorophyll in presence of H_2O_2 (Matile, 1980; Ponmeni & Mukherjee, 1997). Peroxidase activity was found to be much higher in senescent than in the young stage of leaves (Mukherjee & Rao, 1993). Enhanced peroxidase activity was associated with an increase in the level of peroxides and free radicals which react with cellular constituents (Brennan & Frenkel, 1977; Sood *et al*., 2006). A decline in APX activity, progressive increase in SOD activity, changes in GR activity and increase in endogenous H_2O_2 were observed in *Hemerocallis* over the senescence period (Chakrabarty *et al.,* 2009). H_2O_2 is reduced by ascorbate peroxidase (APX) with the consequent oxidation of ascorbate to dehydroascorbate while catalase converts H_2O_2 into H_2O and O_2. The increase in SOD activity over the senescing period could be due to the over-expression of genes induced by H_2O_2 accumulation (Hossain *et al*., 2006). In *Gladiolus* petals, the decrease in APX activity was assumed to be the prerequisite for flower senescence resulting in an increase of the endogenous H_2O_2 level. In *Iris,* APX and SOD activity decreased by the time when the tepals showed wilting, while CAT activity increased and GR activity exhibited no change (Bailly *et al*., 2001). In daylily, the decline in APX and CAT activity along with LOX action resulted in high H_2O_2 endogenous levels during senescence (Barber & Thompson, 1980; Fukuchi-Mizutani *et al*., 2000; Sood *et al*., 2006).

Lipoxygenase also mediated the oxidation of polyunsaturated fatty acids and production of free radicals (Hildebrand & Hymowitz, 1982). However, the well-defined enzymatic antioxidant defense system (superoxide dismutase, SOD; ascorbate peroxidase, APX; glutathione reductase, GR) protect them against these deleterious effects by scavenging ROS. As senescence advanced, the membrane lipid peroxidation caused membrane leakage (Barber & Thompson, 1980; Fukuchi-Mizutani *et al*., 2000; Sood *et al*., 2006; Chakrabarty *et al.,* 2009) by the action of LOX and release free radicals.

The elevation in protease activity is among the important changes associated with the sepal senescence of *Consolida ajacis* flowers (Shahri & Tahir, 2011b). Proteolytic enzymes have been divided

into several different groups depending on specific site at which they cleave target protein. Most common are cysteine proteases. Cpase are closest functional homologue to caspases in senescent plant tissue and commonly functional during petal senescence (Wagstaff *et al*., 2002). Cpase was upregulated during petal senescence in daylily flowers (Guerrero *et al*., 1998). Proteases are mainly classified into two categories- exo and endoproteases depending upon the cleavage site in protein. Endoproteases include cystiene-, serine- and aspartic- named after amino acid cleavage site in protein. As many as nine cysteine proteases have been isolated from senescing *Petunia* petals. In transgenic *Petunia* petals, expression of 4 cysteine proteases genes was delayed resulting in delay of petal wilting (Jones *et al*., 2005).

The upregulation of various nucleases such as RNases and DNases also increases during senescence in many cut flowers (Winkenbach, 1970; Lesham *et al*., 1986; Panavas *et al*., 2000; Canetti *et al*., 2002; Lers *et al*., 2006). The bifunctional nucleases which are able to degrade both RNA and DNA have also been identified. Their activities and the levels of mRNA encode those increases during plant senescence (Canetti *et al*., 2002; Perez-Amador *et al*., 2000). Degraded nuclear DNA indicated laddering of DNA when fragments of DNA were placed on a gel found in a number of flowers like *Alstroemeria* and gladiolus (Wagstaff *et al*., 2003; Yamada *et al*., 2003). The mRNA abundance of DNase genes was also observed during senescence in petals of *Hemerocallis* (Panavas *et al*., 1999), *Narcissus* (Hunter *et al*., 2004) and *Petunia* (Langston *et al*., 2005). This part showed the formation of reactive oxygen species during stress conditions and upregulation or downregulation of various enzymes to regulate the senescence processes. Reports have confirmed that data are much less conclusive on the actual role of oxidative stress and the protective enzymatic systems with their corresponding isoenzymes in relation to progression of flower senescence in plants. However, more detailed work is needed to address this theme.

ETHYLENE

Ethylene is the major promoter of flower senescence in ethylene sensitive flowers, coordinating senescence pathways and floral abscission (Woltering & Van Doorn, 1988; Trobacher, 2009). A visible sign of senescence in ethylene sensitive flower is accompanied by sudden, transient increase in respiration resulting in burst of endogenously produced ethylene, coordinating the senescence pathways and upregulation of genes for enzymes required for senescence (Kende, 1993; Jones *et al*., 2005; Narumi *et al*., 2006; Ichimura *et al*., 2009). The genes for enzymes include S-adenosylmethionine synthase, β-glucosidase, β-galactosidase, aspartic proteases, nucleases, asparagine synthetase etc. (Woodson *et al*., 1992; Eason *et al*., 2000; Wagstaff *et al.,* 2002; Narumi *et al*., 2006). Ethylene biosynthesis pathway starts with conversion of S-adenosylmethionine (SAM) to ACC by upregulation of SAM synthase,

ACC synthase and ACC oxidase during ethylene sensitive petal senescence (Bufler *et al*., 1980; Jones, 2004; Hoeberichts *et al*., 2007). Antisense techniques prolonged life of cut carnation flowers by inhibiting the conversion of ACC to ethylene by decreasing level of ACC oxidase mRNA (Savin *et al*., 1995; Kosugi *et al*., 2002). In flowers of several species such as *Petunia,* carnation and orchids, senescence is mediated by pollination with evolution of ethylene following contact between pollen and stigmatic surface. Ethylene produced from pollinated stigma has been shown to be translocated via style and ovary to carnation petals triggering ethylene synthetic genes contributing to senescence (ten Have & Woltering, 1997; Shibuya *et al*., 2000; Nukui *et al*., 2004; Satoh *et al*., 2005). Strong evidence for an important role for the gynoecium in carnation petal senescence was observed after gynoecium removal by hand by delaying senescence as petal ethylene unable to reach to normal ethylene level in absence of gynoecium. In unpollinated ethylene sensitive petal senescence, cells become more sensitive to their basal ethylene production, which might be related to decrease in cytokinnin activity in petals. Exposure to ethylene at 0.5 $\mu L\ L^{-1}$ or higher concentrations for 24 h markedly accelerated flower senescence, indicating that *Gentiana scabra* flowers are highly sensitive to ethylene (Shimizu-Yumoto & Ichimura, 2012). Ethylene is also known to be involved in the abscission of flower parts in plants such as *Delphinium* (Van Doorn, 2002; Ichimura *et al*., 2009). Overexpression of gene encoding isopentenyltransferase (*ipt*) results in high level of cytokinins extending life of petals in transgenic *Petunia* flowers. When these *ipt*-overexpressing flowers were treated with ethylene, mRNA abundance of cysteine protease gene remained low for a considerable period of time (Chang *et al*., 2003). Treatments with 1-aminocyclopropane- 1-carboxylic acid (ACC), a precursor of ethylene biosynthesis, enhanced senescence of *Hibiscus rosa-sinensis* L. flowers (Trivellini *et al.,* 2011). Eisinger (1977) and Van Staden (1995) observed cytokinin treatment delayed rise in ethylene production through decrease in ethylene sensitivity in carnation petals. Application of indole-3-acetic acid (IAA) hastened rise in ethylene production and petal wilting while it can reduce ethylene in some tissues like abscission zone (Van Staden, 1995). Abscisic acid (ABA) was also observed to enhance ethylene production and hastened petal wilting (Mayak & Dilley, 1976). If gynoecia are removed, however, induction of ethylene no longer occurs and wilting has been reported. Therefore, it is concluded that ABA acts as an inducer of ethylene only through gynoecium (Shibuya *et al*., 2000; Nukui *et al*., 2004). Application of GA_3 delayed rise in ethylene production and postponed petal wilting. In ethylene insensitive petal senescence, signal might be endogenous, from petal cells or may not require hormones as intermediate signals. ABA treatment in *Hemerocallis* hastened time of visible senescence (Panavas *et al*., 1998). Treatment of GA_3 as a vase solution increased petal life span in cut daffodil

flowers (Hunter *et al*., 2004). Depression of enzyme activities and gene expression of ACC synthase and ACC oxidase were observed in cut carnation flowers under high-temperature conditions (Yangkhamman *et al*., 2007). Exogenous ethylene influences flower opening of cut roses (*Rosa hybrida*) by regulating the genes encoding ethylene biosynthesis enzymes (Nan *et al*., 2005) and increases water loss, anthocyanin degradation, ethylene production and decreases vase life in *Dendrobium* orchids depending on cultivars (Almasi *et al.,* 2012). *Petunia* x *hybrida* over-expressing the antisense BoACS1 gene (ACC synthase) or the antisense BoACO1 gene (ACC oxidase) from broccoli showed reduced ethylene biosynthesis and delay of flower senescence (Huang *et al*., 2007). These evidences have supported involvement of both ethylene dependent and independent pathways that lead to senescence of floral parts especially of the petals. Regarding the pattern of senescence in ethylene insensitive flowers, the data so far accumulated is scanty and more elaborate work are required to understand the ethylene independent pathway and its execution.

ABSCISIC ACID (ABA)

Another important plant hormone involved in flower senescence is ABA which accelerates senescence processes in many cut flowers (Wei *et al*., 2003; Hunter *et al*., 2004). ABA participates in endogenous regulation of senescence processes in rose flowers (Halevy & Mayak, 1981; Panavas *et al*., 1998; Hunter *et al*., 2004), and this may be due to conversion of carotenoids to ABA. ABA accelerates senescence of cut roses by promoting petal growth and respiration, thus decreasing the carbohydrate level in the petals and triggering the chain of metabolic processes leading to aging in rose flowers (Borochov *et al*., 1976). ABA content decreased during bud development and increased during senescence. Several fold increase in ABA concentration was observed during the later stages of senescence which was found to be associated with a drastic reduction of flower water potential and water uptake (Kumar *et al*., 2008a; Arrom & Munne-Bosch, 2012b). The ABA content during flower development has a well-defined trend that is common in many plant species such as squash, four o'clock, daylily and daffodil (Panavas *et al*., 1998; Hunter *et al*., 2004). ABA may act by increasing ethylene sensitivity as observed by exogenous application in *Hibiscus* flowers (Trivellini *et al*., 2007, 2011), suggesting that both hormones are involved in flower senescence. A direct relation between petal ABA concentration and longevity was also observed; the higher the ABA concentration at harvest, the shorter the subsequent vase life (Muller *et al*., 1999). In perianth of daffodils, exogenously applied ABA enhanced the premature accumulation of senescence associated transcripts in petals indicating that ABA induced the transcripts independent of ethylene which was reduced by adding GA_3 in vase solution (Hunter *et al*., 2004). In daylily, exogenously applied ABA causes a loss of differential membrane permeability and increase in lipid

peroxidation and ion leakage (Woltering & Van Doorn, 1988).

ABA-accelerated senescence appears to be mediated through induction of ethylene synthesis, since it is not seen in flowers pretreated with ethylene (Mayak & Dilley, 1976; Ronen & Mayak, 1981; Müller *et al*., 1999). This is because daylily flowers are ethylene-insensitive (Lay-Yee *et al*., 1992), ABA presumably induces flower senescence independently of ethylene (Panavas *et al*., 1998). During senescence of daffodil flowers, however, Hunter *et al*. (2002) reported that although ABA accumulated in the tepals as they senesced, it did not appear to play a signaling role in natural senescence. The increase in ABA concentrations in the tepals occurred after the induction of senescence-associated genes. They concluded that the increase in ABA content was most likely a consequence of the cellular stresses that occurred during senescence and suggested that the hormone does not trigger senescence but may help drive the process to completion. It is clear that ABA is considered as most promising growth regulator which promotes senescence and also inducer of ethylene in ethylene insensitive species but proper mechanism is not known how it induces ethylene. Involvement of ABA in both ethylene-sensitive and ethylene-insensitive flower senescence regulates distinct mechanisms which have not been fully elucidated as yet.

APPROACHES TO ENHANCE POSTHARVEST LIFE OF CUT FLOWERS

Various pre-harvest, harvest and post-harvest factors also influence the postharvest quality and longevity of cut flowers (Halevy & Mayak, 1979). The important pre-harvest factors include; cultivar, light, temperature and mineral nutrients in soils. Physical damage due to pests, diseases besides and certain physiological disorders can markedly reduce the cut flower quality. Time of harvest also influences the postharvest life of cut flowers. Flowers harvested in the afternoon retain higher amount of storage and last longer than those harvested in the morning. Harvesting of flowers is done at immature stage when they are transported at long distance and for local markets, harvested at mature stage (Bhattacharjee & De, 2003). Several postharvest factors such as temperature, relative humidity, light and ethylene, CO_2 and O_2 concentration in the environment also influence longevity of cut flowers (Salunkhe *et al*., 1990). Therefore, post-harvest quality of ornamental plants is a crucial aspect to be considered for successful commercialization. The knowledge obtained through the study of petal PCD may be applied to the objective of producing flowers with a longer vase life (Zhou *et al*., 2005). This goal may be achieved by preserving flower freshness during their transport, by designing new holding solutions of sugars (sucrose, glucose, trehalose, etc.) hormones, bactericides, ethanol, mineral ions and metabolic inhibitors or their combinations and by genetically modifying the flowers

through the introduction of useful genes inducing longest postharvest life. Controlled atmospheres (CA) or modified atmospheres (MAP) are the recent technologies for perishable horticultural products. MAP is an inexpensive technique involving the use of polymeric films to create a low oxygen and high carbon dioxide atmosphere within the package in order to reduce physiological changes and quality loss. It works on the same principle as controlled atmosphere while controlled atmosphere (CA) require precise control on atmospheric gases and more costly in use. But these technologies of MAP and CA are not in much practice for the ornamentals.

Carbohydrate Metabolism and Effect of Exogenous Sugars

Metabolites play a crucial role in the regulation of developmental processes as well as in response to biotic and abiotic stresses in plants (Wingler & Roitsch, 2008). Sugar provides not only energy source but also molecules controlling metabolism, development and gene expression in prokaryotes and eukaryotes (Kumar *et al*., 2008b). The reduction in sugar status is among the important changes associated with the sepal senescence of *Consolida ajacis* L. flowers. Therefore, post-harvest life is strongly dependent on the carbohydrate status and acceptable amount of metabolic sugars that affect rate of senescence (Ho & Nichols, 1977). Senescence causes loss of complex carbohydrate and transient accumulation of soluble sugars (Buchanan-Wollaston *et al*., 2003; Sood *et al*., 2006). Some petals contain starch and fructan. In *Chrysanthemum* petals, both polysaccharides were degraded during petal expansion (Trusty & Miller, 1991). Young petals that reported to contain high starch concentration include *Tradescantia reflexa* (Horie, 1961); *Lilium* (Bieleski *et al*., 2000). Waithaka *et al*. (2001) reported transfer of carbohydrate from senescent lower florets to those developed acropetally during development of inflorescence. The content of sugars in the petal tissues increased during the flower opening period and then declined during senescence in *Ranunculus asiaticus* L. (Shahri & Tahir, 2011a). Tirosh and Mayak (1988) reported that α-amylase plays an important role in mechanism of petal opening and regulates the appearance of senescence syndrome. Glucose derived from starch granules are hydrolysed via β-amylase to maltose which is exported from chloroplast, as substrate for transglucosylation reaction, producing glucose and glucosylated acceptor molecule (Smith *et al*., 2005). Starch is present in form of granules composed of branched polymers; most of these are amylopectin-an α-1, 4, α-1, 6 linked polymer (Zeeman *et al*., 2002). The level and translocation of carbohydrates are considered as main factor affecting the development of rose flower and also as a factor affecting postharvest life of cut flowers (Khayat & Zieslin, 1989). SPS (Sucrose phosphate synthase) also plays a key role in conversion of triose phosphate to sucrose in source leaves or may be subjected to coarse control by demand of sink tissues that cause sucrose accumulation

and observed highest level of SPS gene expression in *Oncidium goldiana* flowers (Li *et al*., 2003).

Sucrose supplementation to cut flowers maintained their ATP levels and the movement ability for a longer time than in those kept in water (Azad *et al*., 2008). Keeping the flower in vase solution containing sucrose has been shown to extend their vase life (Ho & Nichols, 1977; Kuiper *et al*., 1995). Inclusion of other sugars as trehalose, mannitol and inositol in vase solution also delayed senescence in tulips (Iwaya-Inoue & Nonami, 2003; Ranwala & Miller, 2009), *Alstroemeria* (Asil & Roein, 2012) and *Gladiolus* (Otsubo & Iwaya-Inoue, 2000; Yamada *et al.*, 2003; Arora & Singh, 2006). Post-harvest application of sucrose has been reported to increase longevity of some important horticultural crops such as carnation (Verlinden & Garcia, 2004). Preservative for consumers include sugars and antimicrobial compounds that inhibit vascular occlusion (Ichimura *et al.,* 2006). At least part of sugar effect could be explained by abundance of *EIL3* mRNA, which is a transcription factor that translates ethylene signals (Hoeberichts *et al*., 2007) and by lower levels of EIL3 protein. The presence of high sugar level was observed to promote proteasomal degradation of EIN3 (Yanagisawa *et al*., 2003). Pun and Ichimura (2003) observed delay in ethylene biosynthesis and decreased insensitivity to ethylene. Sugars can also delay senescence in ethylene sensitive petals. In *Sandersonia*, the effect of exogenous sugar on senescence was accompanied by a delay in expression of genes involved in fatty acid and protein remobilization (Eason *et al*., 2002). Sucrose treatment showed varied responses in different flowers of same family. Sucrose at 0.05 and 0.2 M significantly enhanced vase life of spikes of *Aquilegia vulgaris* L. and *Consolida ajacis* L. respectively, while it is found ineffective in enhancing vase life in *Ranunculus asiaticus* (Shahri *et al*., 2010). Sucrose addition to the vase solution exerts an effect on flower opening and senescence in cut lily flowers by altering the hormonal balance of several floral tissues among other factors (Arrom & Munne-Bosch, 2012a). Sugar was also found to have stimulatory effect in cut sweet pea flowers (Ichimura & Suto, 1999), *Eustoma* (Cho *et al*., 2001) and roses (Hayat *et al*., 2012) in delaying senescence. Hence, sugar metabolism plays its role actively during senescence stages, transporting mainly sucrose through the phloem. It is clear that exogenous sugars delay time of wilting symptoms during senescence; however, it is often not clear to what extent applied sugars serve to improve petal water relations by increasing the level of osmotic solutes or to delay cell death. Measurements about considerable quantities of sugars present at the time of petal wilting may also have not been detailed due to the following reasons: (a) various tissues in a petal are at different stages of senescence, (b) the cytoplasm and the vacuole may have different sugar levels, and (c) sugars are again formed at some stages during senescence. Furthermore, research is needed in this aspect of breakdown and synthesis of sugar at cellular or tissue as well as at molecular level.

Biocides

Pure water used in flower containers soon becomes contaminated with bacteria and fungi which multiply on plant tissue or debris. Microorganisms in water can cause physical plugging of cut stem and release toxic metabolites. They can evolve damaging levels of ethylene and induce hypersensitive response resulting in PCD (Alvarez, 2000). The organism responsible for production of substances such as tannins can block the conducting vessels of the stem. Some chemical substances (known as biocides) have been found helpful in keeping post-harvest life of cut flowers. 8-hydroxyquinoline (8-HQ) and 8-hydroxyquinolinecitrate (8-HQC) are commonly used biocides. They lower the pH of holding solution preventing vascular blockade of many cut flowers including cut roses (Van Doorn & Perik, 1990). 8-HQ has been known to possess strong antimicrobial properties that eliminate vascular blockage and enhance water uptake so as to maintain water balance by reducing transpiration from flower tissue (Rogers, 1973; Jowker, 2005). Other commonly used disinfectants include STS (silver thiosulphate), dichlorphen, hypochlorite and quaternary ammonium compounds (Ueyama & Ichimura, 1998; Muriithi & Ouma, 2011). A solution containing 8-HQC and sucrose is routinely used to prolong vase life in cut flowers (Lukaszewska & Skutnik, 2003). The maximum vase life and flower diameter was recorded when the cut spikes were immersed in a solution containing sucrose 2% + 8 HQC – 200 ppm + $AgNO_3$ 50 ppm in all the tuberose cvs. Vaibhav, Mexican Single, Shringar, Suvasini and Prajwal (Sudagar *et al*., 2010). Knee (2000) observed that various concentrations of biocides in a solution containing 0.2 g L^{-1} citric acid and 10 g L^{-1} glucose were found effective on cut roses (*Rosa hybrida* L., 'Classy'), *Alstroemeria pelegrina* L. and carnation (*Dianthus caryophyllus* L.). Elgimabi and Ahmed (2009) and Tsegaw *et al*. (2011) also reported the best result in enhancing vase life in cut roses and carnation (Edrisi *et al*., 2012) using biocides as preservatives. Pulse treatment with HQS plus sucrose for 12 h is the most effective for improving pigmentation and use as a commercial cut flower preservative solution to delay flower senescence, enhance quality, and prolong the vase life of sweet pea (Elhindi, 2012). From this part, it is concluded that biocides or disinfectants are the important preservative to prevent growth of bacteria which results in plugging of conducting vessels for proper aeration and water uptake for better postharvest performance of cut flowers. Some disinfectants based on cetrimide and chlorhexidine are phytotoxic. Therefore, freely available, cheaper and safe materials for use to plant like sodium and calcium hypochlorite can be used. Some fungicides may also be used but there effect on vase life is found to be negative. Public concern over health and environmental issues associated materials and non-chemical methods such as use of citric acid may prove to be best for better vase life of cut flowers.

Role of Plant Growth Regulators

Auxins are needed for the initiation of floral primordia. Modifications in the auxin levels may cause abortion or different flower forms (Cheng & Zhao, 2007; McSteen, 2010). Auxins have been found to delay the senescence of cut flowers like carnation and *Petunia* (Halevy & Mayak, 1981). In addition, treatment with the synthetic auxin such as 1-naphthaleneacetic acid (NAA) is useful to reduce abscission of flower buds in roses (Halevy & Kofranek, 1976) and drop of flower-bracts in bougainvillea (Chang & Chen, 2001) and enhance postharvest life and chlorophyll b in cut *Alstroemeria hybrida* (Bagheri *et al.,* 2013). In *Theobroma cacao* flowers, a single application of NAA at anthesis, anticipates petal wilting but prevents flower abscission (Aneja *et al*., 1999; Hasenstein & Zavada, 2001). Application of IAA is also found to hasten the rise in ethylene production and petal wilting in cut carnation flowers (Van Staden, 1995). In carnation petals, a transient increase was observed in the mRNA abundance of an Aux/IAA gene (Hoeberichts *et al.,* 2007). In potted bougainvillea postproduction, auxins delay bract and flower abscission (Meir *et al*., 2007; Gago & Monteiro, 2011). The combination of NAA and AVG (Aminoethoxyvinylglycine, an ethylene inhibitor) extended the inflorescence vase life and longevity of opened flowers more than AVG or NAA alone. The combination also increased the number of open flowers and kept the relative fresh weight of flower stems high. Therefore, application of NAA with AVG is a highly effective treatment for improving the postharvest life of cut *Eustoma* flowers (Yumoto & Ichimura, 2010). The auxins such as IAA and NAA strongly promoted elongation and opening. An inhibitor of auxin transport (2, 3, 5-triiodobenzoic acid, TIBA) and an inhibitor of auxin effects [α-(p-chlorophenoxy)-isobutyric acid; PCIB] inhibited elongation and opening, suggesting that endogenous auxins are among the regulators of the pedicel and ovary elongation and thus of flower opening in *Iris* (Van Doorn *et al.,* 2013).

Gibberellins are mostly used and proven growth regulators in horticulture. Most commonly used one is GA_3. Kohl and Kofraneck (1957) were among the first to investigate the possible use of gibberellins in floricultural crops. According to Eason (2002), treatment of gibberellic acid, a component of certain preservative solutions has been found to delay the onset of tepal fading and wilting in *Sandersonia aurantiaca* flowers and enhanced the longevity, chlorophyll content and superoxide dismutase activities in leaf and flower samples in *Alstroemeria* (Nouri *et al*., 2012). The use of Accel ($BA+GA_{4+7}$) at 25 mg L^{-1} BA has been reported to improve flower opening in *Alstroemeria* (Muthui *et al*., 2001). Brackmann *et al*. (2005) evaluated the effects of GA_3 on three varieties of chrysanthemums and noted the promotion of senescence of both leaves and flowers. The application of GA_3 in the field did not reduce or retard the senescence process in chrysanthemum 'Faroe' (Vieira *et al*., 2010). This author also

studied the biochemical changes in post-harvest chrysanthemum 'Faroe' submitted to different concentrations of GA_3 applied in the field and observed increase in the level of polyamines. A concentration of 10 mg L^{-1} GA_{4+7} can be used to prolong vase life, delay leaf senescence and enhance post-harvest quality of *Alstroemeria* cut flowers during their transport to market (Muthui *et al*., 2006). Gerbera cut flowers held in GA_3 at concentration of 2.5, 5 or 7.5 mg L^{-1} had significantly higher water content in flower heads and stems, hence maintaining flower turgidity, reduction in bent neck and flower senescence and increased flower quality after 14 days of holding compared with control (Emongor, 2004). Post-harvest application of GA_3 (50 ml L^{-1}) with sucrose (50 g L^{-1}) reported to improve the fresh weight, concentration of petal sugar, activities of SOD and decreased LOX activity which delayed petal senescence and enhanced vase life of gladioli (Singh *et al*., 2008). According to Kim and Miller (2009), spray containing GA_{4+7} plus BA might be of commercial value in enhancing postharvest quality of tulip flowers. But concentration over 50mg L^{-1} can lead to unwanted early senescence of mature cut tulip flowers, below this concentration would be most useful for achieving maximum delay in tepal senescence. Abadi (2010) studied the effects of different concentrations of GA_3 on growth and flowering of rose (*Rosa hybrida* cv. Poison) and found that 200 mg L^{-1} GA_3 at pre-harvest stage improved stalk length, fresh weight and yield in rose.

Eisinger (1977) proposed that cytokinins are natural anti-senescence factors and their declining levels account for triggering increased ethylene production. Feeding carnation flowers with 6-methyl purine, an inhibitor of cytokinin oxidase/dehydrogenase, resulted in increased life span of petals suggesting that ethylene promotes inactivation of cytokinins and facilitates the senescence process (Taverner *et al*., 2000). Chang *et al*. (2003) confirmed the role of cytokinins in flower senescence using transgenic approach. The transgenic plants over expressing IPT gene under the SAG12 promoter was found to exhibit significant delay in flower senescence and corresponding increase in the cytokinin content and less sensitivity to ethylene suggesting that the regulation of flower senescence involves the interactive operation of cytokinins and ethylene. Hoeberichts *et al*. (2007) have reported the increase in mRNA abundance of two genes encoding cytokinin oxidase/dehydrogenase during carnation petal senescence which was found to accelerate cytokinin breakdown and promote corolla senescence. Cytokinin action in plant tissue is dependent upon the type of cytokinin; one type of cytokinin occurs naturally in plants and includes zeatin, dihydrozeatin and isopentenyl adenine. Similarly, BA supplied in vase solutions extended vase life in *Grevillea* 'Sylvia' inflorescences (Setyadjit *et al*., 2004) and in *Gerbera jamesonii* Bolus ex. Hook cv. Yanara (Jabeen *et al.,* 2008). Application of 25 and 50 mg L^{-1} BA reduced

the weight loss, chlorosis and anthocyanin degradation in *Eustoma* flowers (Asil & Karimi, 2010). Thidiazuron (TDZ), a phenylurea compound with cytokinin like activity has been found to improve *Iris* flower opening and longevity (Macnish *et al*., 2010). BA effectively delayed leaf yellowing and also tepal senescence in tulips (Van Doorn *et al*., 2011). However, BA produced browning of lower stem end. This was prevented by inclusion of Ca^{2+} in solution. Taken together, it is concluded that commercial regulation of plant growth and development relies heavily on the use of synthetic plant growth regulators (PGR). Concern over the impact of these chemicals on human health and the environment has already limited their use and may limit their availability in the future. Another novel approach to modulate the action of phytohormones is by manipulation at the molecular level. Antisence ACC synthase genes blocking ACC production, or with a gene encoding an enzyme that enhance ACC breakdown, has been reported recently in tomatoes transformed plants which resulted in much reduced ethylene production and delayed fruit ripening. Similar technologies will certainly be used in the future to modify production, transport, degradation and activity of PGR.

Ethanol

Alcohols from methanol to hexanol have been tested but only ethanol has been reported to have positive response in enhancing vase life in many cut flowers. Ethanol reduces or inhibits ethylene production by preventing activity of ACC oxidase to convert ACC to ethylene. Mechanism of action depends upon the concentration of ethanol. At low concentration, ethanol is converted in to acetaldehyde that inhibits formation of ethylene. But at high concentration, it showed negative effects on cell membrane by disrupting cell permeability. Thus, it is proposed that ethanol prevents action of ethylene and penetrating into cell membrane by binding at ethylene binding sites. Ethanol has been found to be effective in increasing vase life of carnation flowers by inhibiting ethylene biosynthesis (Heins & Blakely, 1980; Wu *et al.*, 1992). Exogenous application of ethanol has been shown to delay senescence in tomatoes (Kelly & Saltveit, 1988) and oat (*Avena sativa*) leaves (Salter & Thimann, 1980). Ethanol prevented climacteric ethylene, inhibited conversion of ACC to ethylene, interfered with the action of ACC-synthase and inhibited formation of ACC (Heins, 1980; Wu *et al.*, 1992). In *Chrysanthemum* flowers, ethanol treatment delayed the senescence of flowers and improved the quality of vase life of bluebonnet racemes (Petridou *et al.,* 2001). Pun *et al*. (1999) reported that ethanol increased the vase life of carnation flowers and cultivars showed varied responses to ethanol treatment with regard to vase life increment. Ethanol (2%), along with 2.5% sucrose, delayed senescence in *Lisianthus* flowers (Farokhzad *et al*., 2005). Continuous treatment with 8% ethanol doubled vase life of 'White Sim' carnation (*Dianthus caryophyllus*) flowers (Wu *et al*., 1992). In addition, 8% and 10% ethanol were also

found to be effective in delaying senescence in bougainvillea flower (Hossain *et al*., 2007). Podd and Staden (2004) stated that ethanol, when applied at low concentration as holding solution extended vase life of cut carnation flowers. They also mentioned that low concentration of either ethanol or acetaldehyde apparently decreased the formation of ethylene by inhibiting action of ACC synthase. Treatment with ethanol delayed petal senescence of flowers, possibly through reduced sensitivity to ethylene in cut *Tweedia caerulea* (Pun *et al*., 2013) and in carnation (Adugna *et al*., 2012). Ethanol and aluminium sulphate treatments had the most important role in the extending longevity as well as water uptake in *Rosa hybrida* cv. Black Magic (Hajizadeh *et al*., 2012). In *Rosa hybrida,* longest vase life and minimum ethylene production were obtained by using ethanol at 6% concentration (Imani *et al*., 2013). Ethanol (2%) has also been reported to delay senescence in *Matricaria parthenium* L. (Kaur & Mukherjee, 2012) and *Calendula officinalis* L. (Kaur & Mukherjee, 2013) by lowering activity of α-amylase, starch degradation, lipid peroxidation and peroxidase activity.

Mineral Ions

Ions like aluminium, boron, cobalt, calcium, copper, nickel, zinc and silver in the form of various salts at appropriate concentrations have been used to improve postharvest performance of various cut flowers (Halevy & Mayak, 1981). Calcium has been used to prolong vase life of many cut bulb flowers such as tulips and to improve the quality of carnation, roses and *Petunia* (Halevy & Mayak, 1981; Torre *et al.*, 1999; Asfanani *et al*., 2008). It delays rose petal senescence by protecting membrane proteins and phospholipids from degradation by reducing ethylene production and maintaining solute transport (Halevy *et al*., 2000). Cytokinin and $CaCl_2$ decreased the senescence percentage of petals in rose cut flowers separately and this reduction was highest at higher concentration of these substances (Mortazavi *et al*., 2007). Combined effect of Ca as a flow resistance reducer and HQS as

Fig.1: *Calendula officinalis* flowers treated with (A) methanol 2%; (B) methanol 4%; (C) methanol 6%; (D) n-butanol 2%; (E) n-butanol 4%; (F) n-butanol 6%; (G) ethanol 2%; (H) ethanol 4%; (I) ethanol 6% and (J) control (DDW) [Courtesy of Kaur & Mukherjee, 2013].

a germicidal agent contributed to improved vase life in rose (Cortes *et al*., 2011). Calcium acting as a second messenger in the signalling pathway leading to the induction of SOD, CAT and APX, thereby, increasing the capacity of these antioxidant enzymes to scavenge more free radicals produced in the course of senescence, leading to decrease in lipid peroxidation and increasing membrane stability, being a component of cell membranes and wall, it may also strengthen both the structures and thus delays membrane deterioration and senescence in gladiolus (Sairam *et al*., 2011).

Aluminium as $AlCl_3$ and $Al_2(SO_4)_3$ in the holding solution has been shown to enhance the quality and longevity of cut flowers such as roses, *chrysanthemum* and tuberose due to effect of Al^{3+} to reduce pH of petal cells and stabilizing the anthocyanins (Gowda & Gowda, 1990). Aluminium sulphate is reported to acidify the holding solution, keep it free of microorganisms and also help in better opening of flower buds, thereby maintaining the freshness of cut roses (Liao *et al*., 2001; Singh *et al*., 2004). Ichimura *et al*. (2006) developed and tested a formulation composed of sugar, germicides and aluminium sulphate that is effective in extending the vase life of cut rose flowers.

Treatment with silver has widely used as preservative measures for cut flowers (Whitehead & De Swart, 1980). Silver is a specific inhibitor of ethylene action and has been found to inhibit ethylene induced ethylene production and respiration (Halevy & Mayak, 1981; Rodriguez *et al*., 1999; Binder *et al*., 2007; Strader *et al.,* 2009). Silver thiosulphate (STS) has been used as an efficient ethylene antagonist and has been shown to increase longevity in tuberose (Abbasi & Asil, 2011). The holding solution containing nano silver and sucrose resulted in the longest vase life, best water content of the leaves and flower buds and highest fresh weight gain in roses (Hesham & Kader, 2012). Silicon and nickel also increased postharvest life by decreasing malondialdehyde conent and ACC oxidase activity in cut rose flowers (Kazemi *et al*., 2012). Maintenance of elevated carbohydrate contents and reduced level of hydrolyzing enzymes exhibited by the flowers under mineral salts and sucrose treatments can be correlated with the delay in senescence and increase in postharvest vase life of *Gerbera* flowers (Wani *et al*., 2012). It has been proved to some extent that mineral salts of most of metals are found to be beneficial for enhancing longevity of cut flowers but the mechanism how they delay senescence is not known. Molecular approach may prove to be beneficial to understand this mechanism. Some metal salts like silver salts are highly toxic therefore, keeping public concern over health and environmental issues in mind, use of these should be avoided and nontoxic, freely available, cheaper and safe to use materials should be taken into consideration.

Metabolic Inhibitors

Ethylene is one of the factors involved in causing senescence and short vase life of

many cut flowers (Ichimura *et al*., 2002) especially in ethylene sensitive flowers. Molecules block ethylene receptors such as cyclopropene (CP), 1-methylcyclopropene (1-MCP) and 3,3-dimethylcyclopropene (3, 3-DMCP) and that block ethylene biosynthesis α-(2-aminooxyvinyl Glycine) (AVG) and aminooxyacetic acid (AOA) have been used to prolong the vase life of ethylene-sensitive flowers (Rattanawisalanona *et al*., 2003; Sisler & Serek, 1997; Cook *et al*., 1985; Fujino *et al*., 1981). A volatile compound, 1-methylcyclopropene (1-MCP), is an inhibitor of ethylene action and appears to be non-toxic. It has been reported that the vase life of various cut flowers such as carnation, *Matthiola*, *Consolida*, *Chrysanthemum*, *Anthirrinum* and *Delphinium*, can be extended by exposure to 1–MCP. Treatment with 1-MCP markedly extended the vase life of cut sweet pea as did that with STS (Ichimura *et al*., 2002). The effect of 1-MCP has been reported to prolong the postharvest life of flowers of *Geranium* and *Gentiana* (Jones *et al*., 2001; Ferrante *et al*., 2006; Shimizu-Yumoto & Ichimura, 2012). Treatment with 60 nL L^{-1} 1-MCP for 3 h with 16.47 days vase life, 2.57 mL g^{-1} fresh weight, 2.41 mL g^{-1} water uptake and 2.667 loss of chlorophyll index was better than other treatments (Abadi *et al.,* 2009). 1-MCP and STS extended the vase life of roses (Chamoni *et al*., 2005) and florets and spikes of cut *Freesia* 'Cordula' (Zencirkiran, 2010). 1-MCP had a strong effect of preventing the abscission of floral buds and open flowers in mini *Phalaenopsis* cultivars (Sun *et al*., 2009). Treatment with 2-aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthase, slightly delayed flower senescence in *Hibiscus syriacus* L. and *Gentiana scabra* (Seo *et al*., 2009; Shimizu-Yumoto & Ichimura, 2012).

Cycloheximide (CHI) is an inhibitor of de novo protein synthesis in plant tissue (Ap Rees and Bryant, 1971). It is a translational inhibitor that increased vase life of daylily (*Hemerocallis*) by inhibiting petal wilting (Lay-Yee *et al*., 1992). CHI delays loss of proteins in *Ipomoea* by inhibiting synthesis of some specific proteases responsible for protein degradation (Sultan *et al*., 2002). Pretreatment of *Ranunculus asiaticus* flowers with 0.05 mM CHI for 1h can be used as an effective treatment to improve postharvest longevity in this flower system (Shahri & Tahir, 2010). Aminooxyacetic acid (AOA), and fluridone, an ethylene and an ABA inhibitor, respectively, extended flower longevity (Trivellini *et al*., 2011).

Polyamines (PA's) have been reported as effective anti-senescence agents that have ability to retard chlorophyll loss, membrane deterioration and increase in RNase and protease activities which help to slow the senescence process (Evans & Malmberg, 1989). The major polyamines comprise putrescine, spermidine and spermine, which either occurs naturally or as free bases or bound to phenolics or other low molecular weight compounds (Galston & Kaur-Sawhney, 1990). Exogenous application of spermidine has been found to transiently delay senescence of *Dianthus caryophyllus* and *Petunia hybrida* flowers which has been implicated to be due to the ability of free

spermidine to bind to the main intracellular constitutive molecules such as DNA and stabilizing their structures (Gul *et al*., 2005; Tassoni *et al*., 2006). On the other way, methyl-jasmonates have been found to accelerate senescence in *Petunia hybrida, Dendrobium* and *Phalaenopsis* (Porat *et al*., 1993, 1995), but in *Petunia inflata*, only an earlier colour change has been reported without any promotion of petal wilting after treatment with methyl-jasmonate (Xu *et al*., 2006). Genes encoding enzymes of the jasmonate biosynthetic pathway have been shown to be specifically expressed in floral organs (ovaries, petals and sepals) and involved in reproductive processes include maturation of anthers and release of mature pollen grains (Avanci *et al*., 2010). The known fact that pollination triggers senescence in various flower systems and these jasmonates promote pollen maturation and release which might prove to be a mechanism for role of jasmonates in flower senescence. The role of jasmonates in the senescence of ethylene-sensitive flower systems is not clear as yet. However, more elaborate work is needed to confirm it. It is well proven that almost all metabolic inhibitors delay senescence by blocking the pathway causing senescence. Among them, some of the inhibitors like 1-MCP and AOA are very expensive that these cannot be used by floricultural industries frequently. As both are non-toxic and very expensive, they have therefore limited applications in the developing countries. Lime and potassium permanganate, which are low-cost materials, can be used to remove carbon dioxide and ethylene, respectively in packages. These absorbers can be incorporated in sachets, labels or closure liners, or can be impregnated into the MAP film.

CONCLUSION AND FUTURE RESEARCH

Floriculture has become one of the important high value agricultural industries in many countries. However, one major obstacle for floricultural industries is an early senescence of flowers. Physiological, biochemical and morphological studies provide guidance to understand the mechanism involved during abrupt changes that occur during natural flower senescence and how it can be overwhelmed. Role of reactive oxygen species and the expression of various enzymes affecting postharvest life of cut flowers must also be well understood to control senescence of cut flowers. Adoption of inexpensive and eco-friendly products as better innovative preservation proved better in long lasting quality and decelerating all senescence promoting events with reference to the flower senescence. Biotechnological tools also contributed to raise superior postharvest traits in case of many varieties of flowers. More research work in this field is needed to make flower senescence phenomenon clear with vast scope of floriculture and use of intensive techniques to maintain them for longer period. The present review has a number of important points that are missing in research from initial to end point: (1) In ultrastructural changes, the role of the tonoplast in cell death, and the cause of its rupture, is one of the challenges

for further research on petal senescence. More detailed studies are needed at cellular or tissue level regarding this aspect; (2) Mechanism of transport and sequestration of pigments like are anthocyanins, carotenoids, and betalains inside the vacuole. Genetic studies may prove to be best tool in producing mutant variety ineffective to environment conditions; (3) Main site of phospholipid degradation and to what extent this phospholipid degradation takes place inside the vacuoles and autophagosomes and to what extent inside the membrane still has become a controversy; (4) Genes involved in degradation of macromolecule and organelle has been identified in screens but comparatively little is known about the genes whose product facilitates nutrient remobilization by degrading all these structures; (5) Less conclusive data are available on the actual role of oxidative stress and role of reactive oxygen species and how the protective enzymatic systems with their corresponding isoenzymes play its role in relation to progression of flower senescence in plants as all the enzymes act collectively; (6) Regarding the pattern of senescence in ethylene insensitive flowers, the data so far accumulated is scanty and more elaborate work is required to understand the ethylene independent pathway and its execution. (7) Use of various preservatives like sugars, biocides, mineral ions, growth hormones and metabolic inhibitors has advantages but related to public concern over health and environmental issues; they are harmful to some extent. Therefore, novel technology by manipulating role of phytohormones at molecular level may solve this problem.

REFERENCES

Abadi, D. H. (2010). Yield and quality management of *Rosa hybrida* 'Poison' with plant growth regulators**.** *American-Eurasian Journal of Agriculture and Environmental Sciences, 8*, 736-740.

Abadi, D. H, Kaviani, B., Hoor, S. S., Torkashvand, A. M., & Zarei, R. (2009). Quality management of cut carnation 'Tempo' with 1-MCP. *African Journal of Biotechnology, 8*, 5351-5357.

Abbasi, J., & Asil, M.H. (2011). Study on prolonging the vase life of tuberose cut flowers (*Polianthes tuberosa* L.). *South Western Journal of Horticulture, Biology and Environment*, *2,* 157-165.

Adugna, B., Belew, D., & Kassa, N. (2012). Effect of pulsing solution on postharvest performance of carnation (*Dianthus caryophyllus*) cultivars. *Trends in Horticultural Research*, *2*, 8-13.

Aleksandrushkina, N. I., Kof, E. M., Seredina, A. V., Borzov, A. A., & Vanyushin, B. F. (2008). Degradation of DNA and endonuclease activity associated with senescence in the leaves of pea of normal and aphyllous genotypes. *Russian Journal of Plant Physiology, 55*, 23-32.

Almasi, P., Mohamed, M.T.M., Ahmad, S.H., Kadir, J., & Mirshekari, A. (2012). Postharvest responses of cut *Dendrobium* orchids to exogenous ethylene. African Journal of Biotechnology, *11,* 3895-3902.

Alvarez, M. (2000). Salicylic acid in machinery of hypersensitive response cell death and disease resistant, *Plant Molecular Biology*, *44*, 429-442.

Aneja, M., Gianfagna, T., & Ng, E. (1999). The roles of abscisic acid and ethylene in the abscission and senescence of cocoa flowers. *Plant Growth Regulation, 27*, 149-155.

Ap Rees, T., & Bryant, J. A. (1971). Effects of cycloheximide on protein synthesis and respiration in disks of carrot storage tissue. *Phytochemistry*, *10*, 1183-1190.

Arora, A., & Singh, V. P. (2004). Cysteine protease gene expression and proteolytic activity during floral development and senescence in ethylene-insensitive *Gladiolus grandiflora*. *Journal of Plant Biochemistry and Biotechnology, 13*, 123–126.

Arora, A., & Singh, V. P. (2006). Polyols regulate flower senescence by delaying programmed cell death in *Gladiolus*. *Journal of Plant Biochemistry and Biotechnology, 15*, 139-142.

Arrom, L., & Munne-Bosch, S. (2012a). Sucrose accelerates flower opening and delay senescence through a hormonal effect in cut lily flowers. *Plant Science, 41*, 88-189.

Arrom, L., & Munne-Bosch, S. (2012b). Hormonal regulation of leaf senescence in *Lilium*. *Journal of Plant Physiology, 169*, 1542-1550.

Asfanani, M., Davarynejad, G. H., & Tehranifar, A. (2008). Effects of pre-harvest calcium fertilization on vase life of rose cut flowers cv. Alexander. Proc. EURASIA Sym. on Quality Management in Postharvest Systems 217. In S. Kanlayanarat *et al*. (Eds.), *Acta Horticulture,* 804, ISHS 2008.

Asil, M. H., & Karimi, M. (2010). Efficiency of benzyladenine reduced ethylene production and extended vase life of cut *Eustoma* flowers. *Plant Omic Journal, 3*, 199-203.

Asil, M. H., & Roein, Z. (2012). Beneficial effect of carbohydrate solutions on postharvest characteristics of cut *Alstroemeria*. *South-Western Journal of Horticulture, Biology and Environment, 3*, 85-98.

Avanci, N. C., Luche, D. D., Goldman, G. H., & Goldman, M. H. S. (2010). Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. *Genetics and Molecular Research, 9,* 484–505.

Avila-Rostant, O., Lennon, A. M., & Umaharan, P. (2010). Spathe color variation in *Anthurium andraeanum* Hort.and its relationship to vacuolar pH. *HortScience, 45*, 1768–1772.

Azad, A. K., Ishikawa, T., Ishikawa, T. Sawa. Y., & Shibata, H. (2008). Intracellular energy depletion triggers programmed cell death during petal senescence in tulip. *Journal of Experimental Botany, 59*, 2085-2095.

Azeez, A., Sane, A. P., Bhatnagar, D., & Nath, P. (2007). Enhanced expression of serine proteases during floral senescence in *Gladiolus*. *Phytochemistry*, *68*, 1352–1357.

Bagheri, H., Hashemabadi,D., Sedaghathoor, S., Zarchini, M., & Eslami, A. (2013). Effect of naphthalene acetic acid (NAA) on vase life, chlorophyll b content and water relation of cut *Alstroemeria hybrida*. *Annals of Biological Research, 4*, 59-61.

Bailly, C., Corbineau, F., & Van Doorn, W. G. (2001). Free radical scavenging and senescence in *Iris* tepals. *Plant Physiology and Biochemistry, 39*, 649-656.

Barber, R. F. & Thompson, J. E. (1980). Senescence-dependent increase in the permeability of liposome prepared from bean cotyledon membranes. *Journal of Experimental Botany*, *31*, 1305-1313.

Bartoli, C. G., Guiamet, J. J., & Montaldi E. R. (1997). Ethylene production and responses to exogenous ethylene in senescing petals of *Chrysanthemum morifolium* RAM cv. Unsei. *Plant Science*, *124*, 15-21.

Bartoli, C. G., Simontacchi, M., Montaldi E. R., & Puntarolo S. (1995). Oxidants and antioxidants during aging of chrysanthemum petals. *Plant Science, 129*, 157–165.

Battelli, R., Lombardi, L., Rogers, H.J., Picciarelli, P., Lorenzi, R., & Ceccarelli, N. (2011). Changes in ultrastructure, protease and caspase-like activities during flower senescence in *Lilium longiflorum. Plant Science, 180,* 716–725.

Bhattacharjee, S. K., & De, L. C. (2003). *Advanced Commercial Floriculture* (Vol. I). Aavishkar (Pub), Jaipur.

Bieleski, R., Elgar, J., & Heyes, J. (2000). Mechanical aspects of rapid flower opening in Asiatic lily. *Annals of Botany*, *86*, 1175-1183.

Bieleski, R. L. (1995). Onset of phloem export from senescent petals of daylily. *Plant Physiology*, *109,* 557–565.

Bieleski, R. L., & Reid, M. S. (1992). Physiological changes accompanying senescence in ephemeral daylily flower. *Plant Physiol*ogy, *98*, 1042-1049.

Binder, B. M., Rodriguez, F. I., Bleecker, A. B., & Patterson, S.E. (2007). The effects of group 11 transition metals, including gold, on ethylene binding to the ETR1 receptor and growth of *Arabidopsis thaliana*. *FEBS Letters, 581*, 5105–5109.

Biran, I., & Halevy, A. H. (1974). Effect of varying light intensities and temperature treatments applied to whole plants, or locally to leaves or flower buds, on growth and pigmentation of 'Baccara' roses. *Physiologia Plantarum*, *31*, 175-179.

Borochov, A., Spiegelstein, H., & Philosoph H.S. (1997). Ethylene and flower petal senescence: interrelationship with membrane lipid catabolism. *Physiologia Plantarum*, *100*, 606-612.

Borochov, A., Tirosh, T., & Halevy, A. H. (1976). Abscisic acid content of senescing petals on cut rose flowers as affected by sucrose and water stress. *Plant Physiology*, *58*, 175-178.

Brackmann, A., Belle, R. A., de Freitas, S. R., & de Mello, A. M. (2005). Vase life of chrysanthemum (*Dendranthema grandiflora*) in gibberellic acid solutions. *Revista Ciesncia Rural, Santa Maria*, *35*, 1451-1455.

Breeze, E., Wagstaff, C., Harrison, E., Bramka, I., Rogers, H., Stead, A., Thomas, B., & Buchanan-Wollaston, V. (2004). Gene expression patterns to define stages of postharvest senescence in *Alstroemeria* petals. *Journal of Plant Biotechnology*, *2*, 155-168.

Brennan, T., & Frenkel, C. (1977). Involvement of hydrogen peroxide in regulation of senescence in pear. *Plant Physiol*ogy, *59*, 411-416.

Brown, J. H., Chambers, J. A., & Thompson, J. E. (1991). Acyl chain and head group regulation of phospholipid catabolism in senescing carnation flowers. *Plant Physiology, 95*, 909-916.

Brown, J. H., Lynch, D. V., & Thompson, J. E. (1987). Molecular species specificity of phospholipid breakdown in microsomal membranes of senescing carnation flowers. *Plant Physiol*ogy, *85*, 679–683.

Brown, J. H., Palyath, G., & Thompson, J. E. (1990). Influence of acyl length composition on the degradation of phosphatidylcholine by phospholipase D in carnation microsomal membranes. *Journal of Experimental Botany*, *41*, 979–986.

Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T., & Pink, D. (2003). The molecular analysis of plant senescence: a genomics approach. *Plant Biotechnology Journal*, *1*, 3–22.

Bufler, G., Mor, Y., Reid, M. S., & Fang, S. F. (1980). Changes in 1-aminocyclopropane-1-carboxylic acid content of cut carnation flowers in relation to their senescence. *Planta, 150*, 439-442.

Butler, R. D., & Simon, E. W. (1971). Ultrastructural aspects of senescence in plants. In Bernad, Strehler, L. (Ed.), *Adv. Gerontol. Res* (73-129). New York: Academic Press Inc.

Canetti, L., Lomaniec, E., Elkind, Y., & Lers, A. (2002). Nuclease activities associated with dark-induced and natural leaf senescence in parsley. *Plant Science, 163*, 873-880.

Chakrabarty, D., Verma, A. K., & Datta, S. K. (2009). Oxidative stress and antioxidant activity as the

basis of senescence in *Hemerocallis* (day lily) flowers. *Journal of Horticulture and Forestry, 1*, 113-119.

Chamoni, E., Khalighi, A. M., Joyce, D. C., Irving, D. E., Zamani, Z. A., Mostofi, Y., & Mohsen, K. (2005). Ethylene and anti-ethylene treatment effects on cut First-Red rose. *Journal of Applied Horticulture*, *7*, 3–7.

Chang, H., Jones, M. L., Banowetz, G. M., & Clark, D. G. (2003). Overproduction of cytokinins in petunia flowers transformed with P_{SAG12}-IPT delays corolla senescence and decreases sensitivity to ethylene. *Plant Physiology, 132,* 2174–2183.

Chang, Y. S., & Chen, H. C. (2001). Variability between silver thiosulfate and 1- naphthaleneacetic acid applications in prolonging bract longevity of potted bougainvillea. *Scientia Horticulturae*, *87,* 217-224.

Chen, Z. H., Walker, R. P., Acheson, R. M., Tecsi, L. I., Wingler, A., Lea, P. J., & Leegood, R. C. (2000). Are isocitrate lyase and phosphoenolpyruvate carboxykinase involved in gluconeogenesis during senescence of barley leaves and cucumber cotyledons? *Plant and Cell Physiology, 41*, 960–967.

Cheng, Y., & Zhao, Y. (2007). A role for auxin in flower development. *Journal of Integrative Plant Biology*, *49*, 99−104.

Cho, M. C., Celikel, F. G., Dodge, L., & Reid, M. S. (2001). Sucrose enhances the postharvest quality of cut flowers of *Eustoma grandiflorum* (Raf.) Shinn. *Acta Horticulturae, 543*, 305–315.

Collette, V. E., Jameson, P. E., Schwinn, K. E., Umaharan, P., & Davies, K. M. (2004). Temporal and spatial expression of flavonoid biosynthetic genes in flowers of *Anthurium andraeanum*. *Physiologia Plantarum*, *122*, 297–304.

Cook, D., Rasche, M., & Eisinger, W. (1985). Regulation of ethylene biosynthesis and action in cut carnation flower senescence by cytokinins. *Journal of American Society of Horticultural Sciences*, *110*, 24-27.

Cornah, J. E., Germain, V., Ward, J. L., Beale, M. H., & Smith, S. M. (2004). Lipid utilization, gluconeogenesis, and seedling growth in *Arabidopsis* mutants lacking the glyoxylate cycle enzyme malate synthase. *Journal of Biological Chemistry*, *279*, 42916–42923.

Cortes, M. H., Frias, A. A., Moreno, S. G., Pina, M. M., Guzman, G. H. D. L. C., & Sandoval, S. G. (2011). The effects of calcium on postharvest water status and vase life of *Rosa hybrida* cv. Grand Gala. *International Journal of Agriculture and Biology*, *13*, 233–238.

Dat, J. F., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., & Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. *Cellular and Molecular Life Science, 57*, 779–795.

De Vetten, N. C., & Huber, D. J. (1990). Cell wall changes during the expansion and senescence of carnation (*Dianthus caryophyllus*) petals. *Physiologia Plantarum, 78*, 447-454.

Dela, G., Or, E., Ovadia, R., Nissim-Levi, A., Weiss, D., & Oren-Shamir, M. (2003). Changes in anthocyanin concentration and composition in 'Jaguar' rose flowers due to transient high-temperature conditions. *Plant Science*, *164*, 333-340.

Dertinger, U., Schaza, U., & Schulze, E. D. (2003). Age-dependence of the antioxidative system in tobacco with enhanced glutathione reductase activity or senescence-induced production of cytokinins. *Physiologia Plantarum, 119*, 19-29.

Desai, R., Patel, R., & Mankad, A. (2012). Petal senescence in cut *Tagetes erecta* L. flowers: Role of phenolics. *International Journal of Science, Environment and Technology*, *1*, 485 – 490.

Eason, J. R. (2002). *Sandersonia aurantiaca*: An evaluation of postharvest pulsing solutions to maximise cut flower quality. *New Zealand*

Journal of Crop and Horticultural Science, *30*, 273-279.

Eason, J. R., Johnston, J. W., de Vre, L., Sinclair, B. K., & King, G. A. (2000). Amino acid metabolism in senescing *Sandersonia aurantiaca* flowers: cloning and characterization of asparagine synthetase and glutamine synthetase cDNAs. *Australian Journal of Plant Physiology*, *27*, 389-396.

Eason, J. R., Ryan, D. J., Pinkney, T. T., & O'Donoghue, E. M. (2002). Programmed cell death during flower senescence: Isolation and characterization of cysteine proteinases from *Sandersonia aurantiaca*. *Functional Plant Biology, 29*, 1055-1064.

Edrisi, B., Sadrpoor, A., & Saffari, V. R. (2012). Effects of chemicals on vase life of cut carnation (*Dianthus caryophyllus* L. 'Delphi') and microorganisms population in solution. *Journal of Ornamental and Horticultural Plants, 2*, 1-11.

Eisinger, W. (1977). Role of cytokinins in carnation flower senescence. *Plant Physiol*ogy, *59*, 707–709.

Elgimabi, M. N. & Ahmed, O. K. (2009). Effects of bactericides and sucrose-pulsing on vase life of rose cut flowers (*Rosa hybrid*). *Botany Research International, 2*, 164-168.

Elhindi, K. M. (2012). Effects of postharvest pretreatments and preservative solutions on vase life longevity and flower quality of sweet pea (*Lathyrus odoratus* L.) *Photosynthetica*, *50*, 371-379.

Elibox, W., & Umaharan, P. (2008). Inheritance of major spathe colours in *Anthurium andraeanum* Hort. is determined by three major genes. *Hort Science*, *43*, 787–791.

Emongor, V. E. (2004). Effect of gibberellic acid on postharvest quality and vase life of gerbera cut flowers (*Gerbera jamesonii*). *Journal of Agrnomy*, *3*, 191-195.

Evans, P. T., & Malmberg, R. L. (1989). Do polyamines have a role in plant development? *BioScience*, *33*, 382–388.

Farokhzad, A., Khalighi, A., Mostofi, Y., & Naderi, R. (2005). Role of ethanol in vase life and ethanol production in cut lisianthus (*Eustoma grandiflorum* Mariachii cv. Blue). *Journal of Agriculture and Social Sciences*, *1*, 309-312.

Ferrante, A., Mensuali-Sodi, A., Serra G., & Tognoni, F. (2006). Evaluation of postproduction performance of *Salvia splendens* potted plants for interiors use. *Acta Horticulturae*, *723*, 415-419.

Fobel, M., Lynch, D.V., & Thompson, J. E. (1987). Membrane deterioration in senescing carnation flowers. *Plant Physiology*, *85*, 204–211.

Fujino, D. W., Reid, M. S., & Yang, S. F. (1981). Effects of aminooxyacetic acid on postharvest characteristics of carnation. *Acta Horticulturae, 113*, 59-64.

Fukuchi-Mizutani, M., Ishiguro, K., Nakayama, T., Utsunomiya, Y., Tanaka, Y., Kusumi, T., & Ueda, T. (2000). Molecular and functional characterization of a rose lipooxygenase cDNA related to flower senescence. *Plant Science*, *160*, 129-137.

Gago, C. M. L., & Monteiro, J. A. (2011). NAA and STS effects on bract survival time, carbohydrate content, respiration rate and carbohydrate balance of potted *Bougainvillea spectabilis* Willd. *Postharvest Biology and Technology, 60*, 235- 243.

Galston, A. W., & Davies, P. J. (1970). *Control mechanisms in plant development*. Englewood Cliffes New Jersey: Prentice Hall.

Galston, A. W., & Kaur-Sawhney, R. (1990). Polyamines in plant physiology. *Plant Physiology, 94,* 406–410.

Gonzalez, A. (2009). Pigment loss in response to the environment: A new role for the WD/ bHLH/

MYB anthocyanin regulatory complex. *New Phytologist*, *182*, 1-3.

Gowda, J. V. N., & Gowda, V. N. (1990). Effects of calcium, aluminium and sucrose on vase life of gladiolus. *Crop Research, 3*, 105-106.

Griesbach, R. J. (1992). Correlation of pH and light intensity on flower color in potted *Eustoma grandiflorum* 'Grise'. *Hort Science, 27*, 817-818.

Griesbach, R. J. (2005). A scientific approach to breeding blue orchids: Exploring new frontiers in search of elusive flower colors. *Orchids* (W. Palm Beach, Fla.), *74*, 376–379.

Guerrero, C., de la Calle, M., Reid, M. S., & Valpusesta, V. (1998). Analysis of the expression of two thiol protease from daylily *(Hemerocallis*) during flower senescence. *Plant Molecular Biol*ogy, *36*, 565-571.

Gul, F., Tahir, I., & Sultan, S. M. (2005). Effect of some polyamines and protein synthesis inhibitors on flower senescence in *Petunia hybrida* Vilm. cv. Tint Cascade. *Oriental Science*, *10,* 117–122.

Hajizadeh, H. S. Farokhzad, A., & Chelan, V. G. (2012). Using of preservative solutions to improve postharvest life of *Rosa Hybrid* cv. Black Magic. *Journal of Agricultural Technology*, *8*, 1801-1810.

Halevy, A., & Mayak, S. (1979). Senescence and postharvest physiology of cut flowers- part 1. *Horticultural Reviews*, *1*, 204–236.

Halevy, A., & Mayak, S. (1981). Senescence and postharvest physiology of cut flower- part 2. *Horticultural Reviews, 3*, 59–143.

Halevy, A. H., Borochov, A., & Torre, S. (2000). Calcium regulation of senescence in rose petals. *Physiologia Plantarum, 107*, 214-219.

Halevy, A. H., & Kofranek, A. M. (1976). The prevention of flower bud and leaf abscission in pot roses during simulated transport. *Journal of American Society Horticultural Sciences, 101*, 658-660.

Harborne, J. B. (1988). The flavonoids: Recent advances. In T. W. Goodwin (Ed.), *Plant Pigments* (299–343). London, UK: Academic Press Ltd.

Hasenstein, K. H., & Zavada, M. S. (2001). Auxin modification of the incompatibility response in *Theobroma* cacao. *Physiologia Plantarum, 112*, 113-118.

Hayat, S., Ul-Amin, N., Khan, M. A., Soliman, T. M. A., Nan, M., Hayat, K., Ahmad, I., Kabir, M. R., & Zhao, L. J. (2012). Impact of Silver thiosulfate and sucrose solution on the vase life of rose cut flower cv. Cardinal. *Advances in Environmental Biology, 6*, 1643-1649.

Heins, R. D. (1980). Inhibition of ethylene synthesis and senescence in carnation by ethanol. *Journal of American Society Horticultural Sciences, 105*, 141-144.

Heins, R. D., & Blakely, N. (1980). Influence of ethanol on ethylene biosynthesis and flower senescence of cut carnation. *Scientia Horticulturae, 13*, 361-369.

Hernandez-Jimenez, M. J., Lucas, M. M., & de Felipe M. R. (2002). Antioxidant defence and damage in senescing lupin nodules. *Plant Physiology and Biochemistry, 40*, 645-657.

Hesham, H., & Kader, A. (2012). Effects of nanosilver holding and pulse treatments, in comparison with traditional silver nitrate pulse on water relations and vase life and quality of the cut flowers of *Rosa hybrida* L. cv. 'Tineke'. *World Applied Sciences Journal*, *20*, 130-137.

Hildebrand, D. F., & Hymowitz, T. (1982). Carotene and chlorophyll bleaching by soyabean with or without seed lipooxygenase-1. *Journal of Agriculture and Food Chemistry*, *76*, 249-253.

Ho, L., & Nichols, R. (1977). Translocation of ^{14}C-sucrose in relation to changes in carbohydrate content in rose corollas cut at different stages of development. *Annals of Botany*, *41*, 227–242.

Hoeberichts, F. A., Van Doorn, W. G., Vorst, O., Hall, R. D., & Van Wordragen, M. F. (2007). Sucrose prevents upregulation of senescence associated genes in carnation petals. *Journal of Experimental Botany, 58*, 2873-2885.

Hopkins, M., Taylor, C., Liu, Z., Ma, F., McNamara, L., Wang, T. W., & Thompson, J. E. (2007). Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytologist*, *175*, 201–214.

Horie, K. (1961). The behavior of the petals in the fading of the flowers of *Tradescantia reflexa*. *Protoplasma, 53*, 377-386.

Hortensteiner S. (2006). Chlorophyll degradation during senescence. *Annual Review of Plant Biology*, *57*, 55-77.

Hossain, A. B. M. S., Zuliana, R., Chandran, S., Mohamed, H. A. M., & Boyce, A. N. (2007). *Bougainvillea* flower longevity and delay abscission as affected by different concentrations of ethanol. *Maejo International Journal of Science and Technology, 2*, 234-238.

Hossain, Z., Mandal, A. K. A. Datta S. K., & Biswas, A. K. (2006). Decline in ascorbate peroxidase activity- a prerequisite factor for tepal senescence in gladiolus. *Journal of Plant Physiology, 163*, 186-194.

Huang, L. C., Lai, U. L., Yang, S. F., Chu, M. J., Kuo, C. I., Tsai, M. F., & Sun, C. W. (2007). Delayed flower senescence of *Petunia hybrida* plants transformed with antisense broccoli ACC synthase and ACC oxidase genes. *Postharvest Biology and Technology, 46*, 47-53.

Hunter, D. A., Steele, B. C., & Reid, M. S. (2002). Identification of genes associated with perianth senescence in daffodil (*Narcissus pseudonarcissus* L. 'Dutch Master'). *Plant Science, 163*, 13–21.

Hunter, D. A., Ferrante, A., Vernieri, P., & Reid, M. S. (2004). Role of abscisic acid in perianth senescence of daffodil (*Narcissus pseudonarcissus* 'Dutch Master'). *Physiologia Plantarum*, *121*, 313–321.

Hunter, D. A., Yi, M. F., Xu, X. J., & Reid, M. S. (2004). Role of ethylene in perianth senescence of daffodil (*Narcissus pseudonarcissus* L. 'Dutch Master'). *Postharvest Biology and Technology, 32*, 269-280.

Ichimura, K. (2010). Post-harvest physiology of cut flowers: progress and future aspects. *Bulletin of National Institute of Floricultural Science, 10*, 11–53 (In Japanese).

Ichimura, K., Shimizu-Yumoto, H., & Goto, R. (2009). Ethylene production by gynoecium and receptacle is associated with sepal abscission in cut *Delphinium* flowers. *Postharvest Biology and Technology*, *52,* 267–272.

Ichimura, K., Taguchi, M., & Norikoshi, R. (2006). Extension of vase life in cut roses by treatment with glucose, isothiazolinic germicide, citric acid and aluminium sulphate solution. *Japan Agricultural Research Quarterly*, *40*, 263-269.

Ichimura, K., Shimizu, H., Hiraya, T., & Hisamatsu, T. (2002). Effect of 1-methylcyclopropene (1-MCP) on the vase life of cut carnation, *Delphinium* and sweet pea flowers. *Bulletin of National Institute of Floricultural Science, 2*, 1-8.

Ichimura, K., & Suto, K. (1999). Effect of time of sucrose treatment on vase life, soluble carbohydrate concentrations and ethylene production in cut sweet pea flowers. *Plant Growth Regulation, 28*, 117-122.

Imani, M. H., Hashemabadi, D., Kaviani, B., & Zarchini, M. (2013). Improving water relations and postharvest quality of cut rose (*Rosa hybrida* L. cv. 'Avalanche') by ethanol. *Annals of Biological Research*, *4*, 256-259.

Irani, N. G., & Grotewold, E. (2005). Light-induced morphological alteration in anthocyanin accumulating vacuoles of maize cells. *BMC Plant Biology*, *5*, 7.

Iwaya-Inoue, M., & Nonami, H. (2003). Effects of trehalose on flower senescence from view point of physical status of water. *Environment Control in Biology*, *41*, 315.

Jabeen, A., Chandrasekhar, R., & Padma, M. (2008). Effect of plant growth regulators on physical changes and microbial count during vase life of cut gerbera flowers (*Gerbera jamesonii* Bolus ex. Hook.). cv. Yanara. *Journal of Ornamental Horticulture*, *11*, 107-111.

Jones, M. L. (2004). Changes in gene expression during senescence. In L.D. Nooden (Ed.), *Plant cell death processes* (pp. 51–71). Amsterdam: Elsevier.

Jones, M. L., Chaffin, G. S., Eason, J. R., & Clark, D.G. (2005). Ethylene sensitivity regulates proteolytic activity and cysteine protease gene expression in petunia corollas. *Journal of Experimental Botany*, *56,* 2733-2744.

Jones, M. L., Kim, E., & Newman, S. E. (2001). Role of ethylene and 1-MCP in flower development and petal abscission in zonal geraniums. *Hort Science*, *36*, 1305–1309.

Jowker, M. M. (2005). Effect of different compounds on microbial population of cut 'Shiraz narcissus' vase solution. V. International Postharvest symposium, ISHS, *Acta Horticulture*, *682,* 1705-1708.

Katsumoto, Y., Fukuchi-Mizutani, M., Kukui, Y., Brugliera, F., Holton, T. A., Karan, M., Nakamura, N., Yonekura-Sakakibara, K., Togami, J., Pigeaire, A., Tao, G.-Q., Nehra, N. S., Lu, C. Y., Dyson, B. K., Tsuda, S., Ashikari, T., Kusumi, T., Mason, J. G., & Tanaka, Y. (2007). Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiology*, *48*, 1589–1600.

Kaur, P., & Mukherjee, D. (2012). Delaying postharvest senescence of *Matricaria parthenium* L. flowers using ethanol, methanol and sucrose. *Journal of tropical plant physiology*, *4*, 1-16.

Kaur, P., & Mukherjee, D. (2013). Senescence regulation by alcohols in cut flowers of *Calendula officinalis* L. *Acta Physiologae Plantarum*, *35,* 1853-1861.

Kawabata, S., Ohta, S. M., Kusuhara, Y., & Sakiyama, R. (1995). Influence of low light intensities on the pigmentation of *Eustoma grandiflorum* flowers. *Acta Horticulturae, 405*, 173-178.

Kazemi, M., Gholami, M., & Aghdasi, S. (2012). Efficiency of silicon, nickel and acetylsalicylic acid reduced senescence and extended vase life of cut rose flowers. *Trends in Applied Sciences Research, 7,* 590-595.

Kelly, M. O. & Saltveit, M. E. (1988). Effect of endogenously synthesized and exogenously applied ethanol on tomato fruit ripening. *Plant Physiology*, *88,* 143-147.

Kende, H. (1993). Ethylene biosynthesis. *Annual Review of Plant Physiol*ogy, *44*, 283-307.

Khayat, E., & Zieslin, N. (1989). Translocation of ^{14}C-carbohydrate content and activity of enzymes of sucrose metabolism in rose petals at different night temperatures. *Physiologia Plantarum*, *76,* 581-585.

Kim, H. J., & Miller, W. B. (2009). GA_{4+7} plus BA enhance postproduction quality in pot tulips. *Postharvest Biology and Technology, 51*, 272–277.

Kishimoto, S., Maoka, T., Nakayama, M., & Ohmiya, A. (2004). Carotenoid composition in petals of chrysanthemum (*Dendranthemarandiflorum* (Ramat). *Phytochemistry*, *65*, 2781–278.

Knee, M. (2000). Selection of biocides for use in floral preservatives. *Postharvest Biology and Technology*, *18*, 227–234.

Knowles, L. Trimble, M. R., & Knowles, N. R. (2001). Phosphorus status affects postharvest

respiration, membrane permeability and lipid chemistry of European seedless cucumber fruit (*Cucumis sativus* L.). *Postharvest Biology and Technology*, *2*, 179-188.

Kohl, H. C. Jr., & Kofraneck, A. M. (1957). Gibberellin on flower crops. *California Agriculture*, *1*, 9.

Kosugi, Y., Waki, K., Iwazaki, Y., Tsuruno, N., Mochizuki, A., Yoshioka, T., Hashiba, T., & Satoh, S. (2002). Senescence and gene expression of transgenic non-ethylene-producing carnation flowers. *Journal of the Japanese Society for Horticultural Science*, *71*, 638–642.

Kuiper, D., Ribot, S. A., Van Reenen, H. S., & Marissen, N. (1995). The effect of sucrose on the flower bud opening of Madelon cut roses. *Scientia Horticulturae*, *60*, 325-336.

Kuiper, D., Van Reenen, H. S., & Ribot, S. A. (1991). Effect of gibberellic acid on sugar transport into petals of 'Madelon' rose flowers during bud opening. *Acta Horticulturae, 298*, 93-95.

Kumar, N., Srivastava, G. C., & Dixit, K. (2008a). Hormonal regulation of flower senescence in roses (*Rosa hybrida* L.). *Plant Growth Regulation*, *55*, 65–71.

Kumar, N., Srivastava, G. C., & Dixit, K. (2008b). Flower bud opening and senescence in roses (*Rosa hybrida* L.) *Plant Growth Regulation, 55*, 81-99.

Langston, B. J., Bai, S., & Jones, M. L. (2005). Increases in DNA fragmentation and induction of a senescence-specific nuclease are delayed during corolla senescence in ethylene-insensitive (etr1-1) transgenic petunias. *Journal of Experimental Botany, 56*, 15–23.

Lay-Yee, M., Stead, A. D., & Reid, M. S. (1992). Flower senescence in daylily (*Hemerocallis*). *Physiologia Plantarum, 86*, 308-314.

Lei, W., Geng-Guo, T., & Tong, L. (2009). Physiological, biochemical and ultrastructures of mesophyll cell changes in a senescing corolla of *Lycoris longituba*. *Journal of Zhejiang Forestry College*, *26,* 498-502.

Lers, A., Sonego, L., Green, P. J., & Burd, S. (2006). Suppression of LX ribonuclease in tomato results in a delay of leaf senescence and abscission. *Plant Physiology*, *142,* 710- 721.

Lesham, Y. Y., Halevy, A. H., & Frenkel, C. (1986). In *Free radical and control of plant senescence* (pp. 100-116). New York: Elsevier.

Leverentz, M. K., Wagstaff, C., Rogers, H.J., Stead, A.D., Chanasut, U., Silkowski, H., Thomas, B., Weichert, H., Feussner, I., & Griffiths, G. (2000). Characterization of novel lipoxygenase-independent senescence mechanism in *Alstroemeria* floral tissue. *Plant Physiology, 130,* 273–283.

Leverentz, M. K., Wagstaff, C., Rogers, H.J., Stead, A.D., Chanasut, U., Sikowski, H., Thomas, B., Weichert, H., Feussner, I., & Griffiths, G. (2002). Characterization of a novel lipoxygenase independent senescence mechanism in *Alstroemeria* floral tissue. *Plant Physiology, 130*, 273-283.

Li, C. R., Zhang, X .B., & Hew, C. S. (2003). Cloning of a sucrose-phosphate synthase gene highly expressed in flowers from the tropical epiphytic orchid *Oncidium goldiana*. *Journal of Experimental Botany, 54***,** 2189–2191.

Liao, L., Lin, Y., Huang, K., & Chen, W. (2001). Vase life of *Eustoma grandiflorum* as affected by aluminum sulfate. *Botanical Bulletin of Academica Sinica*, *42*, 35-38.

Louda, J. W., Liu, L., & Baker, E. W. (2002). Senescence and death-related alteration of chlorophylls and carotenoids in marine phytoplankton. *Organic Geochemistry*, *33*, 1635-1653.

Lukaszewska, A. J., & Skutnik, E. (2003). *Przewodnik florysty*. Wyd. SGGW, War szawa, pp. 127-138.

Macnish, A. J., Jiang, C., & Reid, M. S. (2010). Treatment with thidiazuron improves opening and vase life of iris flowers. *Postharvest Biology and Technology*, *56*, 77–84.

Matile, P. (1980). Catabolism of chlorophyll: involvement of peroxidase. *Zeitschrift Fur Pflanzenphysiologie*, *107*, 269-279.

Matile, P., & Winkenbach, F. (1971). Function of lysosomes and lysosomal enzymes in senescing corolla of morning glory (*Ipomoea purpurea*). *Journal of Experimental Botany*, *23*, 759-771.

Mayak, S., & Dilley, D. (1976). Effect of sucrose on responses of cut carnation flowers to kinetin, ethylene and abscisic acid. *Journal of American Society and Horticultural Sciences, 101*, 583–585.

Mazza, G. (2007). Anthocyanins and heart health. *Annali dell'Istituto Superiore di Sanita, 43*, 369-374.

McSteen, P. (2010). Auxin and Monocot Development. *Cold Spring Harbor Perspectives in Biol*ogy, *2,* a001479.

Meir, S., Kochanek, B.. Glick, A., Salim, S., Lers, A., Burd, S., Philosoph-Hadas, S., & Weiss D. (2009). Reduced petal pigmentation in lisianthus (*Eustoma grandiflorum*) flowers under low light conditions is associated with decreased expression of anthocyanin biosynthesis genes. ISHS *Acta Horticulturae*, 877: VI International Postharvest Symposium.

Meir, S., Salim, S., Chernov, Z., & Philosoph-Hadas, S. (2007). Quality improvement of cut flowers and potted plants with postharvest treatments based on various cytokinins and auxins. *Acta Horticulturae*, *755*, 143-156.

Meng, X., & Wang, X. (2004). Regulation of flower development and anthocyanin accumulation in *Gerbera hybrida. The Journal of Horticultural Science and Biotechnology*, *79*, 131-137.

Moalem-Beno, D., Tamari,G., Leitner-Dagan, Y., Borochov, A., & Weiss, D. (1997). Sugar-dependent gibberellin-induced chalcone synthase gene expression in *Petunia* corollas. *Plant Physiology, 113*, 419-424.

Moehs, C. P., Tian, L., Osteryoung, K. W., & Penna, D. (2001). Analysis of carotenoid biosynthetic gene expression during marigold petal development. *Plant Molecular Biology, 45*, 281–293.

Mortazavi, N., Naderi, R., Khalighi, A., Babalar, M., & Allizadeh, H. (2007). The effect of cytokinin and calcium on cut flower quality in rose (*Rosa hybrida* L.) cv. Illona. *Journal of Food, Agriculture and Environment*, *5,* 311-313.

Mukherjee, D., & Rao, K. U. M. (1993). Alternation pattern of Hill activity, peroxidase activity and sugars of pigeonpea during maturation and senescence. *Plant Physiology and Biochemistry, 20*, 45-48.

Müller, F., Adori, C., & Sass, M. (2004). Autophagic and apoptotic features during programmed cell death in fat body of the tobacco hornworm (*Manduca sexta*). *European Journal of Cell Biology, 83*, 67-78.

Müller, R., Stummann, B. M., Andersen, A. S., & Serek, M. (1999). Involvement of ABA in postharvest life of miniature potted roses. *Plant Growth Regulation*, *29*, 143–150.

Muriithi, K., & Ouma. G. (2011). The effect of sugar and hypochlorite on the vaselife of cut roses and carnations. *Journal of Animal and Plant Sciences*, *11*, 1394-1397.

Muthui, T. M., Emonger, V. E., & Hutchinson, M. J. (2001). Effect of Accel on vase life and postharvest quality of cut *Alstroemeria* (*Alstoremeria aurantiaca*) cut flowers. *African Journal of Science and Technology, 2*, 82-88.

Muthui, T. M., Emongor, V. E., & Hutchinson, M. J. (2006). The effects of gibberellin$_{4+7}$ on the

vase life and flower quality of *Alstroemeria* cut flowers. *Plant Growth Regulation, 48*, 207–214.

Nan, M., Lei, C., Wangjin, L., Hui, T., & Junping, G. (2005). Exogenous ethylene influences flower opening of cut roses (*Rosa hybrida*) by regulating the genes encoding ethylene biosynthesis enzymes. *Science in China Ser. C Life Sciences*, *48*, 434-444.

Narumi, T., Sudo, R., & Satoh, S. (2006). Cloning and characterization of a cDNA encoding a putative nuclease related to petal senescence in carnation (*Dianthus caryophyllus* L.) flowers. *Journal of the Japanese Society for Horticultural Science*, *75*, 323-327.

Nielsen, K. M., Lewis, D. H., & Morgan, E. R. (2003). Characterization of carotenoid pigments and their biosynthesis in two yellow flowered lines of *Sandersonia aurantiaca* (Hook.). *Euphytica*, *130*, 25–34.

Nouri, N., Abdossi, N., & Boojar, M. (2012). The effect of gibberellic acid and benzyladenine on post harvest quality and vase life of *Alstroemeria* cultivar Mayfair cut flowers with enzymatic assay. *International Journal of Agriculture: Research and Review*, *2*, 1025-1031.

Nowak, J., & Rudnicki, R. M. (1990). *Postharvest Handling and Storage of Cut Flowers, Florist Greens and Potted Plants (1st Edn.)*. Portland: Timber Press. ISBN-13: 978-0881921564, 210p.

Nukui, H., Kudo, S., Yamashita, A., & Satoh, S. (2004). Repressed ethylene production in the gynoecium of long-lasting flowers of the carnation 'White Candle': role of the gynoecium in carnation flower senescence. *Journal of Experimental Botany*, *55*, 641–650.

O'Donoghue, E., Eason, J.R., Somerfield, S. D. & Ryan, D. A. (2005). Galactosidases in opening, senescing and water-stressed *Sandersonia aurantiaca* flowers. *Functional Plant Biology, 32*, 911–922.

Otsubo, M., & Iwaya-Inoue, M. (2000). Trehalose delays senescence in cut gladiolus spikes. *Horticultural Sciences, 35*, 1107-1110.

Pak, C., & Van Doorn, W. G. (2005). Delay of Iris flower senescence by protease inhibitors. *New Phytologist, 165*, 473–480.

Panavas, T., LeVangie, R., Mistler, J., Reid, P. D., & Rubinstein, B. (2000). Activities of nucleases in senescing daylily petals. *Plant Physiology and Biochemistry*, *38*, 837–843.

Panavas, T., Pikula, A., Reid, P. D., Rubinstein, B., & Walker, E. L. (1999). Identification of senescence-associated genes from daylily petals. *Plant Molecular Biology*, *40,* 237–248.

Panavas, T., Reid, P. D., & Rubinstein, B. (1998). Programmed cell death of daylily petals: activities of wall-based enzymes and effects of heat shock. *Plant Physiology and Biochemistry*, *36,* 379–388.

Perez-Amador, M. A., Abler, M. L., De Rocher, E. J., Thompson, D. M., Van Hoof, A., Le Brasseur, N. D., Lers, A., & Green, P. J. (2000). Identification of BFN1, a bifunctional nuclease induced during leaf and stem senescence in Arabidopsis. *Plant Physiology*, *122*, 169-179.

Petridou, M., Voyiatzi, C., & Voyiatzi, D. (2001). Methanol, ethanol and other compounds retards leaf senescence and improve the vase life and quality of cut chrysanthemum flowers. *Postharvest Biology and Technology*, *23,* 79-83.

Pistelli, L., Perata, P., & Alpi, A. (1992). Effects of leaf senescence on glyoxylate cycle enzyme activities. *Australian Journal of Plant Physiology, 19*, 723-729.

Plaut, Z., Zieslin, N., Grawa, A., & Gazit, M. (1979). The response of rose plants to evaporative cooling: Flower production and quality. *Scientia Horticulturae, 11*, 183-190.

Podd, L. A., & Staden, J. V. (2004). The role of ethanol and acetaldehyde in flower senescence and fruit

ripening. *Journal of Plant Growth Regulation*, *26*, 183-189.

Ponmeni, G., & Mukherjee, D. (1997). Kinetin induced alteration in senescence of red gram leaves. *Indian Journal of Plant Physiology, 2*, 250-251.

Porat, R., Borochov, A., & Halevy, A. (1993). Enhancement of *Petunia* and *Dendrobium* flower senescence by jasmonic acid methyl ester is via the promotion of ethylene production. *Plant Growth Regulation*, *13*, 297–301.

Porat, R., Reiss, N., Atzorn, R., Halevy, A. H., & Borochov, A. (1995). Examination of the possible involvement of lipoxygenase and jasmonates in pollination-induced senescence of *Phalaenopsis* and *Dendrobium* flowers. *Physiologia Plantarum, 94* 205–210.

Prochazkova, D., Sairam, R. K., Srivastava, G. C., & Singh, D. V. (2001). Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Science*, *161*, 765-771.

Pun, U. K., & Ichimura, K. (2003). Role of sugars in senescence and biosynthesis of ethylene in cut flowers. *Japan Agricultural Research. Quarterly*, *37*, 219-224.

Pun, U. K., Niki, T., & Ichimura, K. (2013). Ethanol reduces sensitivity to ethylene and delays petal senescence in cut *Tweedia caerulea* flowers. *Plant Growth Regulation, 69*, 125–130.

Pun, U. K., Rowe, R. N., Rowarth, J. S., Barnes, M. F., Dawson, C. O., & Heyes, J. A. (1999). Influence of ethanol on climacteric senescence in five cultivars of carnation. *New Zealand Journal of Crop and Horticultural Sciences*, *27*, 69-77.

Ranwala, A. P., & Miller, W. B. (2009). Comparison of the dynamics of non-structural carbohydrate pools in cut tulip stems supplied with sucrose or trehalose. *Postharvest Biology and Technology*, *52*, 91-96.

Rattanawisalanona, C., Ketsa S., & Van Doorn W. G. (2003). Effect of aminooxyacetic acid and sugars on the vase life of *Dendrobium* flowers. *Postharvest Biology and Technology*, *29,* 93-100.

Rodriguez, F. I., Esch, J.J., Hall, A. E., Binder, B. M., Schaller, G. E., & Bleecker, A. B. (1999). A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. *Science*, *283*, 996–998.

Rogers, H. J. (2006). Programmed cell death in floral organs: How and why do flowers die? *Annals of Botany*, *97*, 309–315.

Rogers, H. J. (2012). Is there an important role for reactive oxygen species and redox regulation during floral senescence? *Plant, Cell and Environment, 35,* 217-233.

Rogers, M. N. (1973). An historical and critical review of postharvest physiology research on cut flowers, *Hort. Science, 8*, 189-194.

Ronen, M., & Mayak, S. (1981). Interrelationship between abscisic acid and ethylene in the control of senescence processes in carnation flowers. *Journal of Experimental Botany*, *32*, 759-765.

Roy, P., Roy, S. P., Mitra, A., & Kulkarni, A. P. (1994). Superoxide generation by lipooxygenase in presence of NADH and NADPH, *Biochimica et Biophysica Acta*, *1214,* 171-179.

Rubinstein, B. (2000). Regulation of cell death in flower petals. *Plant Molecular Biology*, *44*, 303-318.

Sairam, R. K., Vasanthan, B., & Arora, A. (2011). Calcium regulates *Gladiolus* flower senescence by influencing antioxidative enzymes activity. *Acta Physiologiae Plantarum, 33*, 1897–1904.

Salter, S. O., & Thimann, K. V. (1980). The influence of aliphatic alcohols on leaf senescence. *Plant Physiology, 66*, 395-399.

Salunkhe, F. B., Bhat, N. R., & Desai, B. B. (1990). Postharvest Biotechnology of Flowers and Ornamental Plants. Berlin, Heidelberg: *Springer-Verlag.*

Sankhla, N., Mackay, W. A., & Davis, T. D. (2005). Corolla abscission and petal color in cut *Phlox* flower heads: Effects of sucrose and thidiazuron Proc. VIIIth IS Postharvest Phys. Ornamentals. In N. Marissen *et al.* (Eds.), *Acta Horticulturae*, 669, ISHS.

Satoh, S., Nukui, H., & Inokuma, T. (2005). A method for determining the vase life of cut spray carnation flowers. *Journal of Applied Horticulture, 7,* 8-10.

Savin, K. W., Baudinette, S. C., Graham, M. W., Michael, M. Z., Nugent, G. D., Lu, C. Y., Chandler, S. F., & Cornish, E. C. (1995). Antisense ACC oxidase RNA delays carnation petal senescence. *HortScience, 30*, 970–972.

Seo, S-G., Kang, S-W., Shim, I-S., Kim,W., & Fujihara, S. (2009). Effects of various chemical agents and early ethylene production on floral senescence of *Hibiscus syriacus* L. *Plant Growth Regulation, 57*, 251–258.

Setyadjit, Joyce, D. C., Irving, D. E., & Simons, D. H. (2004). Effects of 6-benzyladenine treatments on the longevity of harvested *Grevillea* 'Sylvia' inflorescences. *Plant Growth Regulation*, *43*, 9-14.

Shahri, W. (2011). Senescence: Concept and Synonyms. *Asian Journal of Plant Sciences*, *10*, 24-28.

Shahri, W., & Tahir, I. (2010). Effect of cycloheximide on senescence and post-harvest performance of *Ranunculus asiaticus* L. flowers. *Pakistan Journal of Botany, 42*, 3577-3585.

Shahri, W., & Tahir, I. (2011a). Flower development and senescence in *Ranunculus asiaticus* L. *Journal of Fruit and Ornamental Plant Research, 19*, 123-131.

Shahri, W., & Tahir, I. (2011b). Physiological and biochemical changes associated with flower development and senescence in *Consolida ajacis* Nieuwl cv. Violet Blue. *Frontiers of Agricultur***e** *in China, 5,* 201–208.

Shahri, W., Tahir, I., Isla-m, S. T., & Ahmad, M. (2010). Response of some ornamental flowers of family Ranunculaceae to sucrose feeding. *African Journal of Plant Sciences, 4*, 346-352.

Shahri, W., Tahir, I., Islam, S.T., & Bhat, M.A. (2011). Physiological and biochemical changes associated with flower development and senescence in so far unexplored *Helleborus orientalis* Lam. cv. Olympicus. *Physiology and Molecular Biology of Plants*, *17*, 33–39.

Shibuya, K., Yoshioka, T., Hashiba, T., & Satoh, S. (2000). Role of the gynoecium in natural senescence of carnation (*Dianthus caryophyllus* L.) flowers. *Journal of Experimental Botany*, *51,* 2067–2073.

Shimizu-Yumoto, H., & Ichimura, K. (2012). Effects of ethylene, pollination, and ethylene inhibitor treatments on flower senescence of gentians. *Postharvest Biology and Technology, 63*, 111–115.

Shoji, K., Miki, N., Nakajima, N., Momonoi, K., Kato, C., & Yoshida, K. (2007). Perianth bottom-specific blue colour development in tulip cv. Murasakizuisho requires ferric ions. *Plant Cell Physiology, 48,* 243–251.

Shvarts, M., Borochov, A., & Weiss, D. (1997). Low temperature enhances petunia flower pigmentation and induces chalcone synthase gene expression. *Physiologia Plantarum, 99*, 67-72.

Singh, A., Kumar, J., & Kumar, P. (2008). Effects of plant growth regulators and sucrose on postharvest physiology, membrane stability and vase life of cut spikes of gladiolus*. Plant Growth Regulation*, *55*, 221–229.

Singh, K., Singh, P. J., & Kumar, R. (2004). Effect of some chemical treatments on keeping quality of cut roses. *Advances in Horticultural Sciences, 18*, 161-167.

Sisler, E. C., & Serek, M. (1997). Inhibitors of ethylene responses in plants at receptor level:

Recent developments. *Physiologia Plantarum*, *100*, 577-582.

Smith, A. M., Zeeman, S. C., & Smith, S. M. (2005). Starch degradation in *Arabidopsis*. *Annual Review of Plant Biology*, *56*, 73-98.

Smith, M., & Butler, R. D. (1971). Ultrastructural aspects in *Cucumis sativus* with particular reference to chromoplasts. *Protoplasma, 73*, 1-13.

Smith, M. T., Saks, Y., & Van Staden, J. (1992). Ultrastructural changes in petals of senescing flowers of *Dianthus caryophyllus* L. *Annals of Botany*, *69*, 277-285.

Sood, S., Vyas, D., & Nagar, P.K. (2006). Physiological and biochemical studies during flower development in two rose species. *Scientia Horticulturae, 108*, 390–396.

Spundova, M., Popelkova, H., Ilik, P., Skotnica, J., Novotny, R., & Naus, J. (2003). Ultrastructural and functional changes in the chloroplasts of detached barley leaves senescing under dark and light conditions. *Journal of Plant Physiology*, *160*, 1051-1058.

Stead, A., & Van Doorn, W. G. (1994). Strategies of flower senescence: a review. In In R. J. Scott and A. D. Stead (Eds.), *Molecular and Cellular Aspect of Plant Reproduction* (pp. 215-238). Cambridge.

Stephenson, P., & Rubinstein, B. (1998). Characterization of proteolytic activity during senescence in day lilies. *Physiologia Plantarum*, *104*, 463-473.

Strader, L. C., Beisner, E. R., & Bartel, B. (2009). Silver ions increase auxin efflux independently of effects on ethylene response. *The Plant Cell*, *21*, 3585–3590.

Sudagar, I. P., Sankarnarayannan, R., & Aruna, P. (2010). Effect of chemicals in increasing the vase life of tuberose cultivars. *The Asian Journal of Horticulture, 4*, 421-423.

Sugawara, H., Shibuya, K., Yoshioka, T., Hashiba, T., & Satoh, S. (2002). Is a cysteine proteinase inhibitor involved in the regulation of petal wilting in senescing carnation (*Dianthus caryophyllus* L.) flowers? *Journal of Experimental Botany*, *53*, 407–413.

Sultan, S. M., Tahir, I., Arif, M., & Farooq, S. (2002). Cycloheximide spray treatment soon after opening prolongs longevity of detached morning glory (*Ipomoea tricolor*) flowers. *Journal of Plant Biology, 29*, 105-118.

Sun, Y., Christensen, B., Liu, F., Wang, H., & Muller, R. (2009). Effects of ethylene and 1-MCP (1-methylcyclopropene) on bud and flower drop in mini *Phalaenopsis* cultivars. *Plant Growth Regulation, 59*, 83–91.

Tassoni, A., Accettulli, P., & Bagni, N. (2006). Exogenous spermidine delays senescence of *Dianthus caryophyllus* flowers. *Plant Biosystematics, 140*, 107–114.

Taverner, E., Lethem, D. S., Wang, J., & Cornish, E. (2000). Inhibition of carnation petal inrolling by growth retardants and cytokinins. *Australian Journal of Plant Physiology*, *27*, 357–362.

Ten Have, A., & Woltering, E. J. (1997). Ethylene biosynthetic genes are differentially expressed during carnation (*Dianthus caryophyllus* L.) flower senescence. *Plant Molecular Biology, 34*, 89–97.

Thompson, J. (1988). The molecular basis for membrane deterioration during senescence. In L.D. Noodén and A.C. Leopold (Eds.), *Senescence and Aging in Plants* (pp. 51–83). New York: *Academic*.

Thompson, J. E., Mayak, S., Shinitzky, M., & Halevy, A. H. (1982). Accelration of membrane senescence in cut carnation flower by treatment with ethylene. *Plant Physiology*, *69*, 859-863.

Tirosh, T., & Mayak, S. (1988). Changes in starch content during development of carnation petals. *Journal of Plant Physiology, 113*, 361-363.

Torre, S., Borochov, A., & Halevy, A. H. (1999). Calcium regulation in senescence in rose petals. *Physiologia Plantarum, 107*, 214-219.

Tripathi, S. K., & Tuteja, N. (2007). Integrated signaling in flower senescence. *Plant signaling and Behavior, 2*, 437-44.

Trivellini, A., Ferrante, A., Lucchesini, M., Mensuali-Sodi, A. Vernieri, P., Tognoni, F., & Serra, G. (2007). Ethylene and abscisic acid interaction during hibiscus (*Hibiscus rosa-sinensis* L.) flower development and senescence. *Advances in Plant Ethylene Reasearch*, *2*, 75-79.

Trivellini, A., Ferrante, A., Vernieri, P., Mensuali-Sodi, A., & Serra, G. (2011). Effects of promoters and inhibitors of ethylene and ABA on flower senescence of *Hibiscus rosa-sinensis* L. *Journal of Plant Growth Regulation, 30,* 175–184.

Trobacher, C. P. (2009). Ethylene and programmed cell death in plants. *Botany, 87,* 757-769.

Trusty, S. E., & Miller, W. B. (1991). Postproduction carbohydrate levels in pot chrysanthemums. *Journal of the American Society for Horticultural Science*, *116*, 1013-1018.

Tsegaw, T., Tilahun, S., & Humphries, G. (2011). Influence of pulsing biocides and preservative solution treatment on the vase life of cut rose (*Rosa hybrida* L.) varieties *Ethiopian Journal of Applied Sciences and Technology, 2*, 1 -18.

Uddin, A. F. M. J., Hashimoto, F., Kaketani, M., Shimizu, K., & Sakata, Y. (2001). Analysis of light and sucrose potencies on petal coloration and pigmentation of lilianthus cultivars (*in vitro*). *Scientia Horticulturae*, *89*, 73-82.

Ueyama, S., & Ichimura, K. (1998). Effects of 2-hydroxy-3-ionene chloride polymer on vase life of rose flowers. *Postharvest Biology and Technology, 14*, 65-70.

Vaknin, H., Bar-Akiva, A., Ovadia, R., Ada Nissim-Levi, A., Forer, I., Weiss, D., & Oren-Shamir, M. (2005). Active anthocyanin degradation in *Brunfelsia calycina* (yesterday–today–tomorrow) flowers. *Planta*, *222*, 19–26.

Van Doorn, W. G. (2002). Effect of ethylene on flower abscission: A survey. *Annals of Botany* (London), *89*, 689-693.

Van Doorn, W. G. (2011). Classes of programmed cell death in plants, compared to those in animals. *Journal of Experimental Botany*, *62*, 4749-4761.

Van Doorn, W. G., & Perik, R. R. J. (1990). Hydroxyquinoline citrate and low pH prevent vascular blockages in stems of cut rose flowers by reducing number of bacteria. *Journal of American Society and Horticultural Sciences*, *115*, 979-981.

Van Doorn, W. G., & Woltering, E. (2004). Senescence and programmed cell death: Substance or semantics? *Journal of Experimental Botany*, *55*, 2147–2153.

Van Doorn, W. G., & Woltering, E. J. (2008). Physiology and molecular biology of petal senescence. *Journal of Experimental Botany*, *59*, 453–480.

Van Doorn, W. G., Dole, I., Celikel, F. G., & Harkema, H. (2013). Opening of *Iris* flowers is regulated by endogenous auxins. *Journal of Plant Physiology*, *170*, 161– 164.

Van Doorn, W. G., Perik, R. R. J., Abadie, P., & Harkem, H. (2011). A treatment to improve the vase life of cut tulips: Effects on tepal senescence, tepal abscission, leaf yellowing and stem elongation. *Postharvest Biology and Technology, 61*, 56-63.

Van Doorn,W. G., Balk, P. A., van Houwelingen, A. M., Hoeberichts, F. A., Hall, R. D.,Vorst, O., van der Schoot, C., & van Wordragen, M. F. (2003). Gene expression during anthesis and senescence in iris flowers. *Plant Molecular Biology, 53*, 845–863.

Van Staden, J. (1995). Hormonal control of carnation flower senescence. *Acta Horticulturae, 405,* 232-239.

Verlinden, S., & Garcia, J. J. V. (2004). Sucrose loading decreases ethylene responsiveness in carnation (*Dianthus caryophyllus* cv. White Sim) petals. Postharvest Biology and Technology, *31*, 305-312.

Vieira, M. R. S., Teixeira da Silva, J. A., Lima, G .P. P., & Vianello, F. (2010). Changes in polyamine, total protein and total carbohydrate content and peroxidase activity during the lifetime of chrysanthemum 'Faroe'. *Floriculture and Ornamental Biotechnology*, *48*, 52.

Voleti, S. R., Singh, V. P., Arora, A., Singh, N., & Kushwaha, S.R. (2000). Physiology of flower senescence in floriculture crops. In A. Hemantaranjan (Ed.), *Advances in Plant Physiology* (pp. 423-439). Jodhpur, India. Jodhpur: Scientific Publishers.

Wagstaff, C., Leverentz, M.K., Griffiths, G., Thomas, B., Chanasut, U., Stead, A. D., & Rogers, H. J. (2002). Cysteine protease gene expression and proteolytic activity during senescence of *Alstroemeria* petals. *Journal of Experimental Botany*, *53*, 233-240.

Wagstaff, C., Malcom, P., Arfan, R., Leverentz, M.K., Griffith, G., Thomas, B., Stead, A.D., & Rogers, H. (2003). Programmed cell death (PCD) processes begin extremely early in *Alstroemeria* petal senescence. *New Phytologist*, *160*, 49–59.

Waithaka, K., Dodge, L. L., & Reid, M. S. (2001). Carbohydrate traffic during opening of gladiolus florets. *Journal of Horticultural Science and Biotechnology*, *76*, 20-24.

Wani, M., Saha, S., Bidwai, J., & Khetmalas, M. (2012). Changes in carbohydrate levels and associated enzyme activities during post harvest vase life of *Gerbera jamesonii* cv. Danalin flowers as influenced by mineral salts. *Journal of Horticulture Letters*, *2*, 08-11.

Wei, Z., Zhang, H., Gu, Z. P., & Zhang, J. J. (2003). Cause of senescence of nine sorts of flowers. *Acta Botanica Sinica, 33*, 429-436.

Weiss, D. (2000). Regulation of flower pigmentation and growth: multiple signaling pathways control anthocyanin synthesis in expanding petals. *Physiologia Plantarum*, *110*, 152-157.

Whitehead, C. S., & De Swardt, G. H. (1980). The inhibitory effect of silver ions on certain metabolic processes after uptake and distribution in different floral parts of carnations. *Agroplantae*, *2,* 61-64.

Wiemken, V., Wiemken, A., & Matile, P. (1976). Physiologie der Blüten von *Ipomoea tricolor* (Cav.): Untersuchungen an abgeschnittenen Blüten und Gewinnung eines Phloemexudates. *Biochemie und Physiologie* der *Pflanzen, 169*, 363-376.

Wilhelmova, N., Domingues, P. M. D. N., Srbova, M., Fuksova, H., & Wilhelm, J. (2006). Changes in non-polar aldehydes in bean cotyledons during aging. *Biologia Plantarum*, *50*, 559-564.

Wills, R., McGlasson, B., Graham, D., & Joyce, D. (1998). Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamentals (4th Edn.). Sydney: UNSW Press, pp. 1-262.

Wingler, A., & Roitsch T. (2008). Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. *Plant Biology* (Stuttg), *1*, 50–62.

Winkenbach, F. (1970). Zum Stoffwechsel der aufbluhenden und Welkenden Korolle der Prunkwide *Ipomea purpurea*. *Berichte* der *Schweizerischen Botanischen Gesellschaft, 80*, 374-406.

Woltering, E. J., & Van Doorn, W. G. (1988). Role of ethylene in senescence of petals- morphological and taxonomical relationships. *Journal of Experimental Botany, 39*, 1605-1616.

Woodson, W. R., Park, K. Y., Drory, A., Larsen, P. B. & Wang, H. (1992). Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. *Plant Physiology*, *99*, 526–532.

Wu, M. J., Zacarias, L., Saltveit, M. E., & Reid, M. S. (1992). Alcohols and carnation senescence. *Horticultural Sciences*, *27*, 136-138.

Xu, Y., Ishida, H., Reisen, D., & Hanson, M.R. (2006). Upregulation of a tonoplast-localized cytochrome P450 during petal senescence in *Petunia inflata*. *BMC Plant Biology,* 6.

Xu, X., Jiang, C. Z., Donnelly, L., & Reid, M. S. (2007). Functional analysis of a RING domain ankyrin repeat protein that is highly expressed during flower senescence. *Journal of Experimental Botany*, *58,* 3623–3630.

Xu, X., Jiang, C., & Reid, M. S. (2008). Functional analysis of a putative ubiquitin ligase that is highly expressed during flower senescence. *Journal of Experimental Botany* (in press).

Yamada, T., Ichimura, K., Kanekatsu, M., & Van Doorn, W. G. (2007). Gene expression in opening and senescing petals of morning glory (*Ipomoea nil*) flowers. *Plant Cell Reports, 26*, 823–835.

Yamada, T., Ichimura, K., & Van Doorn, W. G. (2006). DNA degradation and nuclear degeneration during programmed cell death in petals of *Antirrhinum*, *Argyranthemum* and *Petunia*. *Journal of Experimental Botany*, *57*, 3543–3552.

Yamada, T., Takatsu, Y., Manabe, T., Kasumi, M., & Marubashi, W. (2003). Suppressive effect of trehalose on apoptotic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus. *Plant Science*, *164*, 213-221.

Yamaguchi, T., Fukada-Tanaka, S., Inagaki, Y., Saito, N., Yonekura-Sakakibara, K., Tanaka, Y., Kusumi, T., & Iida, S. (2001). Genes encoding the vacuolar Na+/H+ exchanger and flower coloration. *Plant Cell Physiology*, *42*, 451–461.

Yanagisawa, S., Yoo, S. D., & Sheen, J. (2003). Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature*, *425*, 521–525.

Yangkhamman, P., Koji, T., Ichimura, K., & Fukai, K. (2007). Depression of enzyme activities and gene expression of ACC synthase and ACC oxidase in cut carnation flowers under high-temperature conditions. *Plant Growth Regulation, 53*, 155–162.

Yao, N., Eisfelder, B. J., Marvin, J., & Greenberg, J. T. (2004). The mitochondrion - an organelle commonly involved in programmed cell death in *Arabidopsis thaliana*. *Plant Journal*, *40*, 596-610.

Yoshida, K. (2003). Molecular regulation of leaf senescence. *Current Opinion in Plant Biology*, *6*, 79-84.

Yoshida, K., Kawachi, M., Mori, M., Maeshima, M., Kondo, M., Nishimura, M., & Kondo, T. (2005). The involvement of tonoplast proton pumps and Na+(K+)/H+ exchangers in the change of petal colour during flower opening of morning glory, *Ipomoea tricolor* cv. Heavenly Blue. *Plant Cell Physiology*, *46*, 407–415.

Yoshida, K., Kondo, T., Okazaki, Y., & Katou, K. (1995). Cause of blue petal colour. *Nature, 373,* 291.

Yoshida, K., Toyama-Kato, Y., Kameda, K., & Kondo, T. (2003). Sepal color variation of *Hydrangea macrophylla* and vacuolar pH measured with a proton-selective microelectrode. *Plant Cell Physiology*, *44*, 262–268.

Yumoto, H., & Ichimura, K. (2010). Combination pulse treatment of 1-naphthaleneacetic acid and aminoethoxyvinylglycine greatly improves postharvest life in cut *Eustoma* flowers. *Postharvest Biology and Technology, 56*, 104–107.

Zamani, S., Kazemi, M., & Aran, M. (2011). Postharvest life of cut rose flowers as affected by salicylic acid and glutamine. *World Applied Science Journal, 12*, 1621-1624.

Zeeman, S. C., Pilling, E., Tiessen, A., Kato, L., Donald, A. M., & Smith, A. M. (2002). Starch synthesis in *Arabidopsis*; granule synthesis, composition and structure. *Plant Physiology*, *129,* 516-529.

Zencirkiran, M. (2010). Effects of 1–MCP (1–methylcyclopropene) and STS (silver thiosulphate) on the vase life of cut *Freesia* flowers. *Scientific Research and Essays*, *5,* 2409-2412.

Zhou, Y., Wang, C.H., Ge, H., Hoeberichts, F. A., & Visser, P. B. (2005). Programmed cell death in relation to petal senescence in ornamental plants. *Acta Botanica Sinica*, *47*, 641–650.

Pertanika J. Trop. Agric. Sci. 37 (2): 203 - 214 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

The Enzyme Activities of Pancreas and Small Intestinal Contents in the Malaysian Village Chicken and Broiler Strains

Khalid K. Kadhim[1], Md Zuki Abu Bakar[1]*, Noordin Mohamed Mustapha[2], Mohd Amin Babjee[2] and Mohd Zamri Saad[2]

[1]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

The digestive enzyme activities of the pancreas and small intestinal contents were examined in two strains of chickens which differ in growth rates from 1 day to 4 months of age. The total body weight (BW) of Commercial Broiler Chicken (CBC) showed marked increase (P<.05) during the experiment, in contrast to Malaysian village chicken (MVC) which only increased slowly over the same period. The pancreas weight of MVC (g/100g BW) was greater (P<.05) than CBC throughout the experiment except for 1 day of age. The pancreatic enzyme activity (unit/g and unit/100g BW) was significantly higher (P<.05) in the CBC, particularly at 10 days old. However, there was no difference between the strains at 20 to 56 days of age for amylase and 120 days for chymotrypsin. The enzyme activity (unit/g) for all intestinal segments increased with age in both strains. In particular, CBC attained a maximal value at 10 days of age for jejunal and ileal trypsin, as well as for duodenal, jejunal and ileal chymotrypsin. Meanwhile, the enzyme activities (unit/100g BW) decreased with age for both the strains. In specific, CBC had a relatively greater enzyme activities (P<.05) at day 1, except for the jejunal trypsin and the chymotrypsin. Thereafter, the relative activities were higher for MVC until the end of the experiment. Although significant differences in the digestive enzyme activities were obtained between the two strains, these differences were generally associated with the differences in body weight. The selection affected the development rate of the synthesis of these enzymes according to the body requirements and biological function and this might affect the digestion and finally the growth rate.

ARTICLE INFO

Article history:
Received: 27 April 2011
Accepted: 22 July 2013

E-mail addresses:
khalidkamkad@yahoo.com (Khalid K. Kadhim),
zuki@upm.edu.my (Md Zuki Abu Bakar),
noordinmm@upm.edu.my (Noordin Mohamed Mustapha),
sm_amin@upm.edu.my (Mohd Amin Babjee),
mzamri@upm.edu.my (Mohd Zamri Saad)
* Corresponding author

ISSN: 1511-3701

Keywords: Enzyme activity, pancreas, intestinal contents, amylase, trypsin, chymotrypsin

INTRODUCTION

In Malaysia, the original Malayan fowls were widespread in villages before the arrival of Europeans in the late 1800s. The present Malaysian native chickens, commonly known as *ayam kampong* (village chickens), are the results of cross-breeding of the Red jungle fowl with mixed exotic domestic breeds brought in by Europeans, mainly the British (Azahan & Zahari, 1983). In general, the indigenous chickens are of small body size, slow growth rate, with plumage of different colours, and of dual-purpose type, as well as variable body conformation and physical characteristics. The chicken's market weight of 1-1.5 kg is reached within four to five months. During the late 1940s, the broiler breeds required 12 weeks to reach the live body weight of 1,800g. Four decades later, this period was reduced by half, with a reduction of essentially 1 day/year to reach this weight, showing no evidence of abatement (Gyles, 1989). Tremendous progress has been made in the selection of broilers for increased growth, feed conversion and carcass quality (Schreiweis *et al*., 2005).

Starch is a major component of poultry feed and its efficient utilization requires the presence of a capable system of appropriate enzymes in the gastrointestinal tracts of the birds (Osman, 1982). The development of supply organs (e.g., pancreas and small intestine) accelerates shortly after hatching in breeds selected for high body weight for which it is essential for secretory activity of the pancreas to achieve maximal growth at an early age. Thus, the functional maturation of these organs is important in the assimilation of feed (Nitsan *et al*., 1991a,b). The lack of pancreatic enzymic hydrolysis in the intestinal lumen decreases the apparent digestibility of the dietary components and reduces growth (Corring & Ourdon, 1977). Many digestive enzymes are synthesized by the exocrine pancreas, stored in the zymogen granules and released into the duodenum (Pubols, 1990). Levels of digestive enzymes in organs and contents of gastrointestinal tract are influenced by genetic stock (O'Sullivan *et al*., 1992). The ratios of digestive enzymes produced by the pancreas of turkeys (Krogdahl & Sell, 1989) and chickens (Pubols, 1991) have been profiled through several variable methods, while age has been determined as a factor (Nitsan *et al*., 1991a; Sell *et al*., 1991). The enzyme activity levels in the pancreas increase with age for relative amylase, total trypsin, total and relative chymotrypsin (O'Sullivan *et al*., 1992).

In the present study, MVC, which is characterized by a slow growth rate, was used to compare with CBC as a breed selected for high growth rate. The experiment was undertaken to determine enzymes (Amylase, Trypsin and Chymotrypsin) activities within both the pancreas and the small intestine (duodenum, jejunum, and ileum). The comparisons were made at days 1, 10, 20, 60 and 120 (4 months) after hatching.

MATERIALS AND METHODS

Experimental animals

The pancreas and intestinal contents were collected from MVC procured from a poultry farm at Universiti Putra Malaysia. The eggs of MVC were obtained from Jenderam Hulu, in Sepang. The villager stocks comprise the local unimproved poultry breeds, including mixed (unspecified) breeds resulting from uncontrolled breeding. The day one old chicks of commercial line (Ross) selected for high body weight were supplied by a private hatchery (Linggi Poultry Farm Sdn. Bhd. C-P lot 1354, 33010, Kuala Kangsar, Perak, Malaysia). The birds were reared in separate cages with a commercial diet and water provided *ad libitum*. Five groups, six males in each, aged one day, 10 days, 20 days, 2 months and 4 months were sacrificed by intravenous injection of sodium pentobarbitone (80 mg/ kg BW) (Mitchell & Smith, 1991).

Sample Collection

Body weight and the pancreas weight of each bird were recorded. The small intestine was divided into duodenum, jejunum and ileum, following the demarcation set by Mitchell and Smith (1990). The intestinal contents of each segment were emptied into tubes by gentle pressure between thumb and fore-finger. All the samples were then placed in liquid nitrogen and stored at -80ºC.

Measurement of the Enzyme Activity

The activity of amylase was measured as described by Howard and Yudkin (1963). The method is based on the decrease in the intensity of blue colour given by the reaction of starch and iodine as the starch is hydrolysed. The homogenate was made by 1/20 wt/vol ice-cold distilled water for pancreatic tissue and 1/4 wt/vol for intestinal chyme, homogenized in a metal mortar. The homogenate was centrifuged at 70.000g for 20 min at 4ºC and the supernatant was stored at -70ºC.

Comparison of colour was made using an absorptiometer with filter maximum absorption at 620 mμ and cells of 1 cm width (Howard & Yudkin, 1963). The enzyme activity was expressed as units of activity, where one unit is defined for amylase as an increase in 10^{-5} extinction at 620 nm/10 min at 37ºC and 10^{-2} for trypsin and chymotrypsin at 410 nm/20 min at 37ºC.

According to Gertler and Nitsan (1970), the activities of pancreatic trypsin and chymotrypsin were determined after the activation of the pancreatic homogenate. The activation was carried out by mixing equal volumes of pancreas supernatant and 1% enterokinase and incubating for 1 h at 37ºC. The purified enterokinase (Sigma-Aldrich) was prepared in 0.1 M-Tris-HCL buffer (tris-hydroxy-methyl amino methane), pH 7.2, containing 0.1M-$CaCl^2$. It was pre-incubated at 37ºC for 30 min and centrifuged at 2000g to remove solid materials.

Trypsin and chymotrypsin activities were estimated with Nα- benzoyl-L- arginine ethyl ester HCL (BAEE) and N-benzoyl-L-tyrosine ethyl ester (BTEE) as the substrates for trypsin and chymotrypsin, respectively

(Gertler & Nitsan, 1970), with BAEE and BTEE obtained from Sigma-Aldrich. The final concentration was 1.25 mM in 3.7 mM-Tris buffer, pH 7.8, 0.6 mM-$CaCl^2$ and 25/mg/ml dimethyl sulfoxide. The reaction proceeded at 30ºC for 30 min and was stopped with (300 ml/l) acetic acid. The colour developed was measured by using the spectrophotometer at 410 nm and the cells of 1 cm width. Activity units were defined as one μ mole substrate hydrolysed /min at 25ºC.

Data of the enzyme activity for pancreatic and intestinal contents were expressed in units per gram of pancreatic tissue or intestinal contents, respectively, and units per 100 g body weight in relative bases. The difference between strains at each age was analyzed by independent t tests.

RESULTS

The pattern of the total body weight for CBC showed a marked increase ($P<.05$) compared to MVC. It increased approximately 4 folds and 5 folds at days 10 and 20 post hatch, respectively, and more than this rate thereafter. For MVC, however, the body weight increased very slowly to the end of the experiment (Fig.1). The absolute weight of the pancreas for the CBC was significantly greater than for MVC throughout the period of the experiment. The pancreas weight for CBC increased approximately 10 folds and 15 folds at days 10 and 20 post hatch, respectively, as compared to the first day, while it was not more than 4 folds and 6 folds for MVC during the same period. The pancreas weight seemed to be constant for CBC after 56 days of age. The weight of pancreas per 100 g body weight showed significantly higher for the CBC at first day and no difference at the remaining days between both strains, while the relative weight of the pancreas declined for both the strains after that. However, MVC showed a significantly greater value at 120 days of age (Fig.2).

In general, the specific activities of pancreatic amylase, trypsin and chymotrypsin increased with age. There

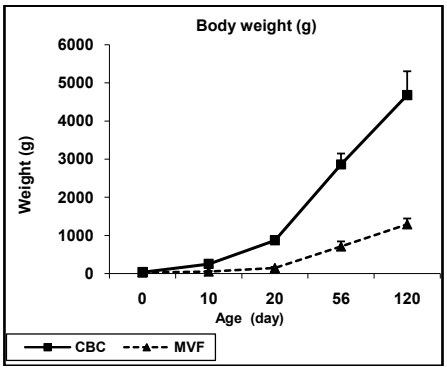


Fig.1: The total body weights of CBC and MVC from 1 day to 4 months of age. Values are means ± SD, N= 6, the difference between breeds at the same age were significant ($P\leq.05$), where, CBC> MVC

was a marked increase at 10 days post hatch for CBC. However, there were minor decreases detected in all these enzymes at 20 days post hatch, particularly for CBC, which increased thereafter until the end of the experiment (Fig.3). Meanwhile, MVC showed gradual increases for all the pancreatic enzymes during the experiment. The pancreatic enzyme activities for CBC were significantly greater than those for MVC at all age groups. The pancreatic amylase activity persistently increased at 120 days for both the strains but showed no difference between them. Meanwhile, the result seemed constant for trypsin and chymotrypsin. When expressed as units of activity per 100 g body weight, the activities of all these pancreatic enzymes increased after hatching, reached a peak at 10 days, when they attained approximately 2 folds, 3 folds and 2 folds increases for amylase, trypsin and chymotrypsin, respectively, for CBC, and 3 folds and 4 folds increases for MVC (Fig.2). The activity of these enzymes showed a decrease after that for both the strains. The relative activities of pancreatic enzymes for CBC were significantly higher than those for MVC, except after 10 day for amylase, when no differences were found between the strains.

As for the contents of the small intestine, the absolute enzyme activity for CBC showed a significantly higher value than MVC throughout the experiment. The enzyme activity of MVC increased gradually throughout the experiment.

For the amylase activity, CBC attained a marked increase at 10 days for all intestinal segments (Fig.4). The increasing rate for both breeds was approximately 2 folds compared to that at 1 day post hatch, and 3 to 5 folds at 120 days, for CBC and MVC, respectively.

When expressed in units of activity per 100g body weight, the activity of amylase at 1 day post hatch represents a significant higher value for CBC in all intestinal segments. After that, the activity curve declines with the age. The relative activity of amylase remains constant to 10 days in

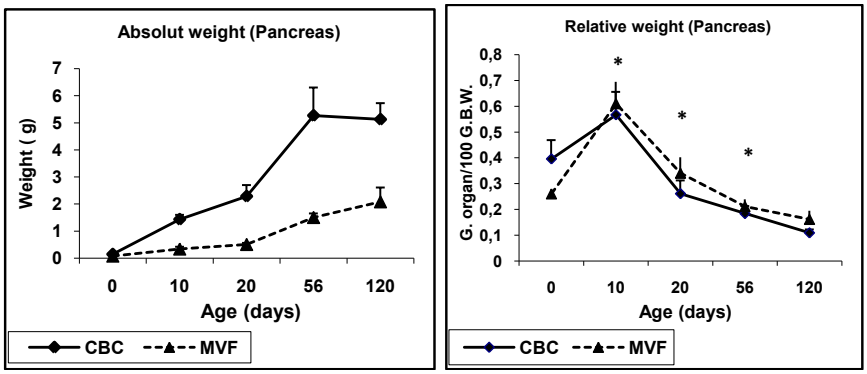


Fig.2: Absolute (a) and relative (b) pancreas weights (g/100 g BW) for CBC and MVC, from 1 day to 4 months of age. Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

MVC, but after that point it decreases until the end of the experiment. Although the relative activity of amylase for MVC was found to be significantly higher, both the strains showed no variances at 10 days for the jejunum (Fig.3).

The pattern of the duodenal trypsin activity was somewhat different than that for amylase in both the strains (Fig.5). At 10 days, there was a marked increase in jejunum and ileum by approximately 2 folds over that at 1 day. However, CBC showed

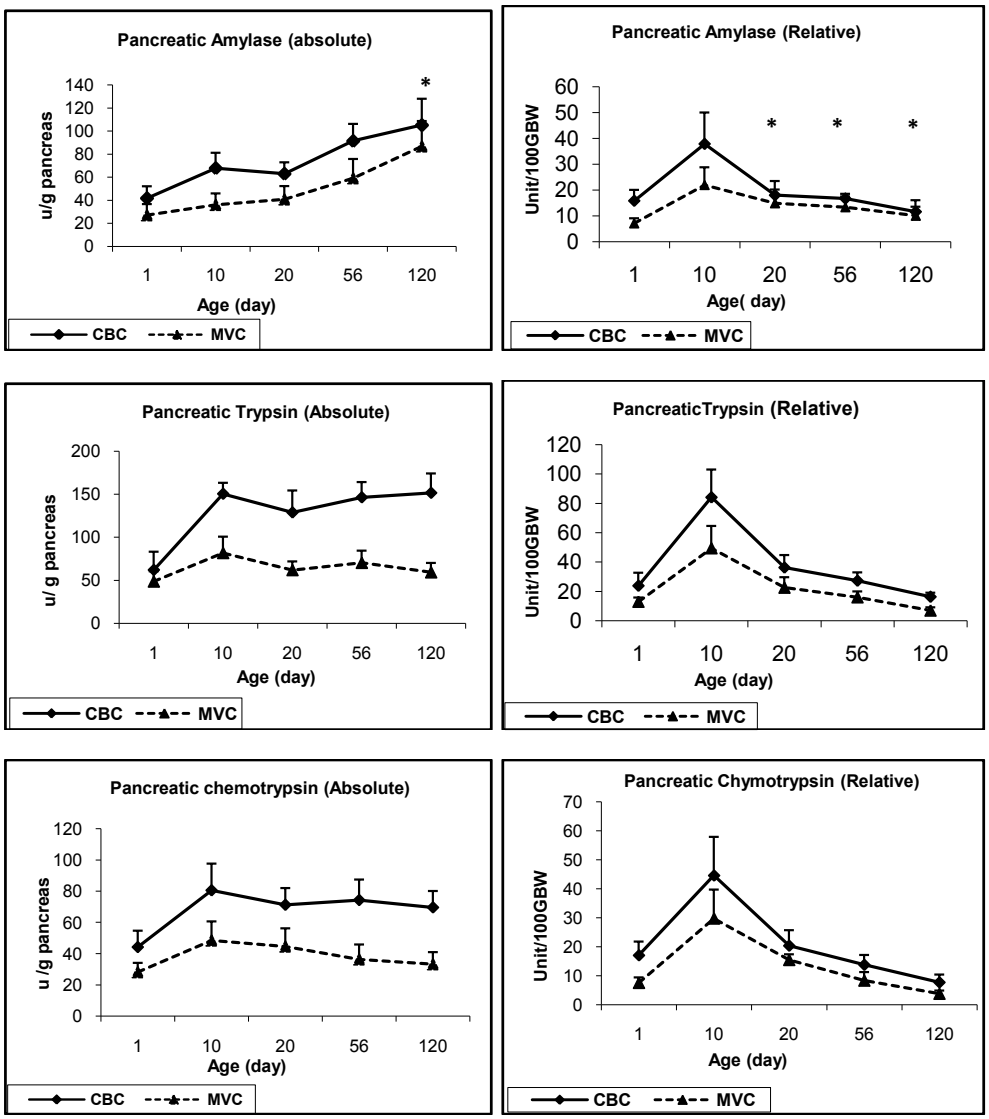


Fig.3: Pancreatic activities of amylase (a, a'), trypsin (b, b') and chymotrypsin (c, c') from 1 day to 4 months of age for CBC and MVC, expressed in units/g organ (left) and units/100g BW (right). Comparisons of the mean are made between lines at each age. Vertical bars represent the SD. N=6. (*) Star superscript indicating not significantly different ($P > .05$).

a decrease in the duodenal trypsin activity at 20 days and at 56 days for the jejunum and ileum, but it increased thereafter. For MVC, the enzyme activity progressed very slowly and declined after 20 days in both jejunum and ileum. The relative activity of trypsin was markedly decreased for both the strains during the experiment (Fig.4). Except for MVC, the jejunum and ileum relative trypsin activity did not change during the first 10 day post-hatch. At 1 day, the relative trypsin activity of CBC was

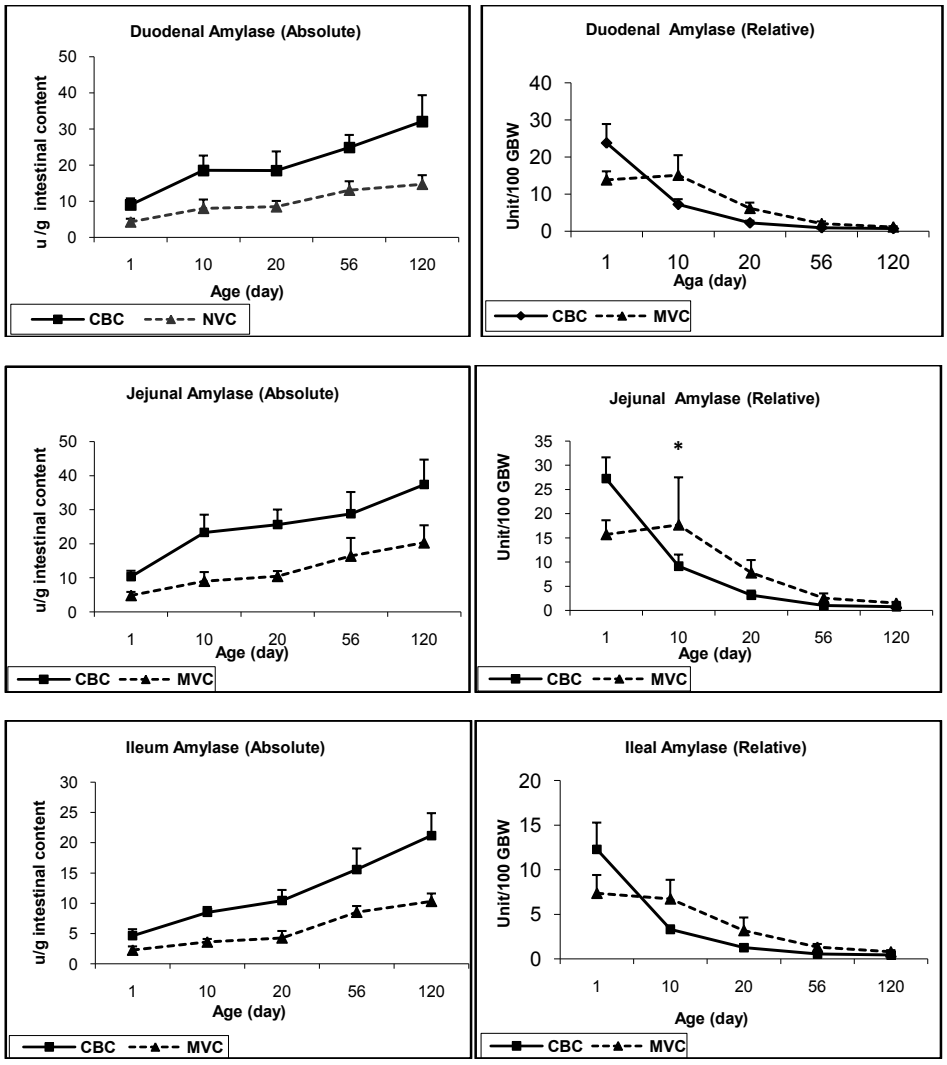


Fig.4: Activities of Amylase in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

significantly higher for the duodenum and ileum, although there was no difference for the jejunum and no difference at 56 day for duodenum and jejunum, while MVC had the greater value during the remaining of the experiment.

The chymotrypsin activity was found to increase by approximately 2 folds for both the strains in all the intestinal segments during the first 10 days. However, it reached 3 folds in the jejunum of CBC. Both the strains showed gradual decreases in the

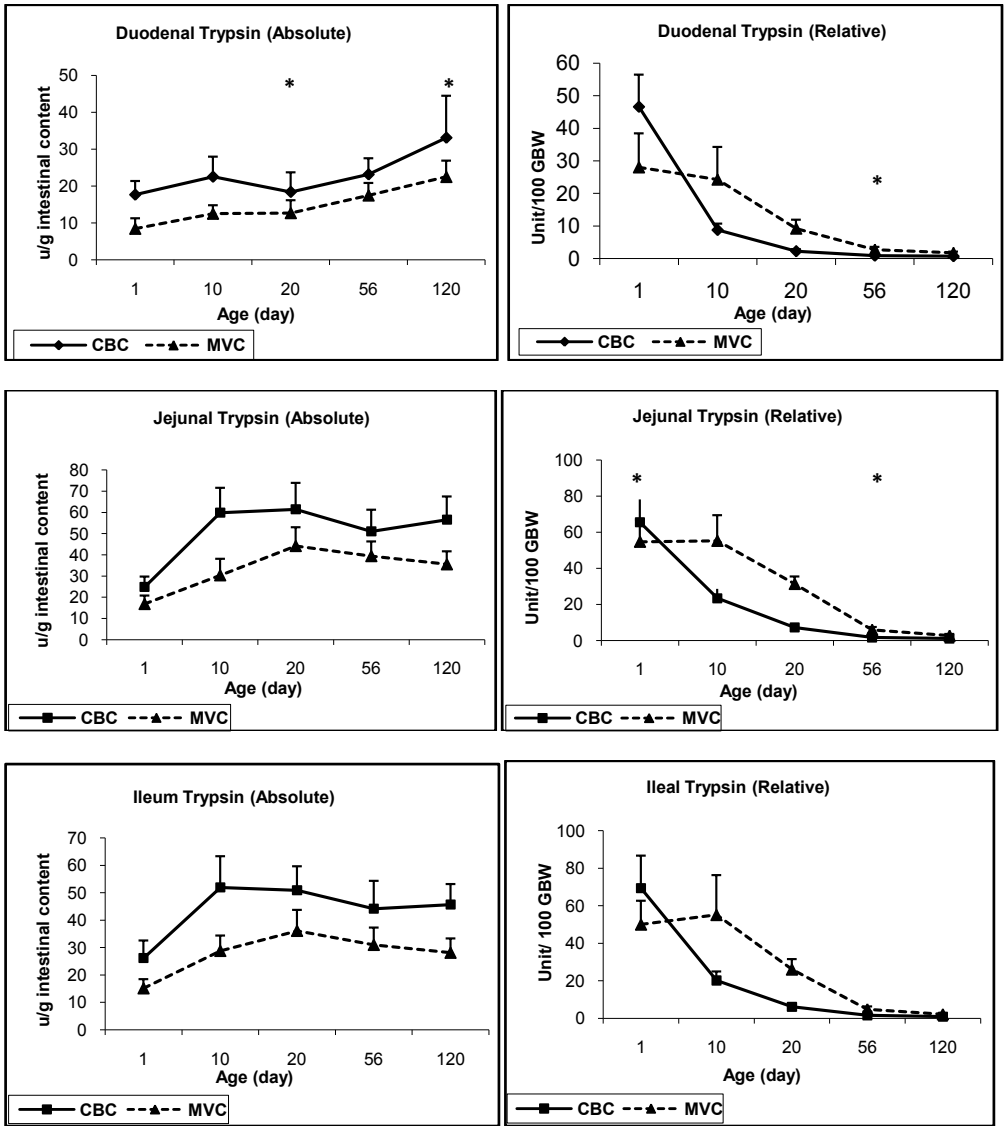


Fig.5: Activities of trypsin in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

enzyme activity with age (Fig.6). The data for the relative chymotrypsin activities showed a decrease during the experiment for all the intestinal segments of both strains, except the relative enzyme activity for MVC showed increases during the first 10 days for all the intestinal segments. In addition, there was no difference between the two strains at the 1 day in jejunum, and at 56 days in duodenum and from this time to the end of experiment in the jejunum. However, MVC showed a significantly higher value during the remaining days of the experiment (Fig.6).

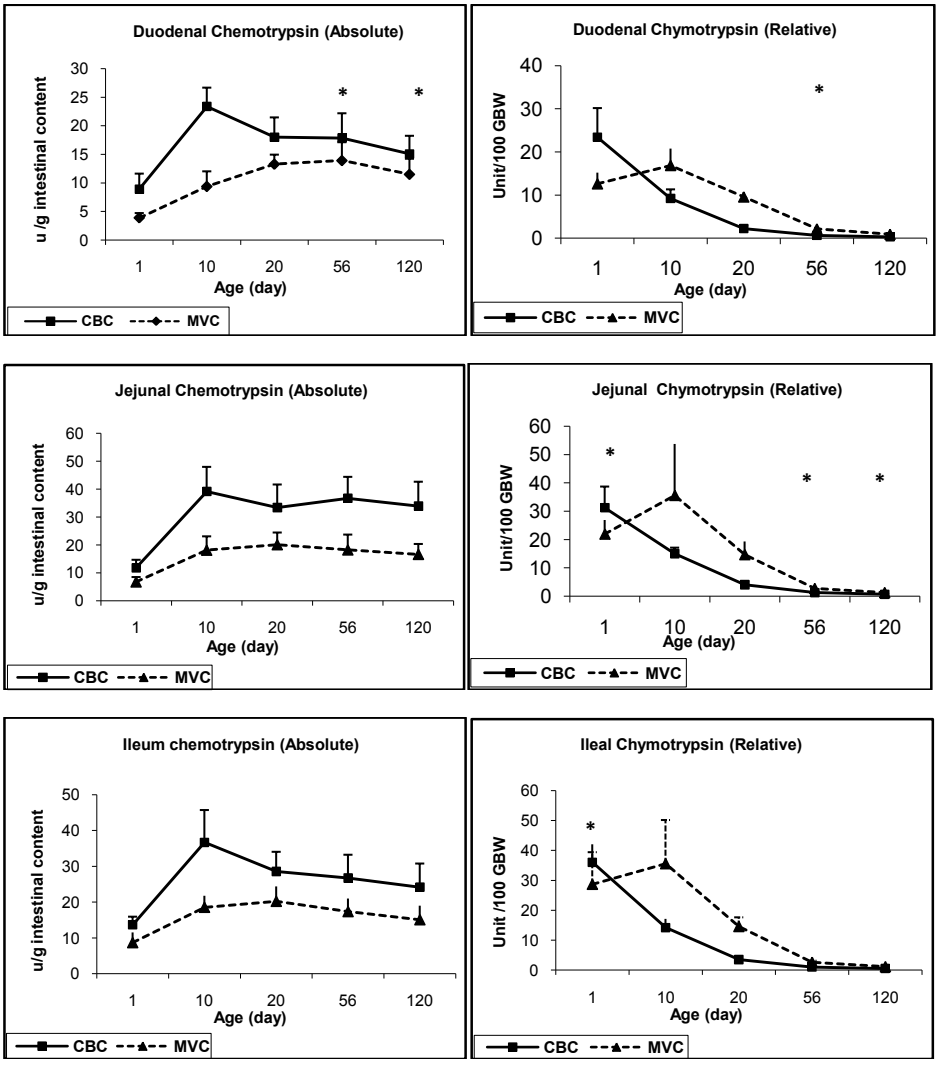


Fig.6: Activities of chymotrypsin, in the contents of the duodenum (a, a'), jejunum (b, b') and ileum (c, c') of CBC and MVC from 1 day to 4 months of age, expressed in units /g (left) and units /100g BW (right). Vertical bars represent SD; when not shown, SD is smaller than the symbol. (*) Star superscript indicating not significantly different ($P > .05$).

DISCUSSION

The difference in the pancreatic weight was clearly observed between the two strains. The relative pancreatic weight constituted 0.4% and 0.2% of body weight on the 1st day of life for CBC and MVC, respectively, increased and peaked at 10 days of age. These differences in the relative pancreatic weight between the two strains might reflect differences in the body growth rate, the pancreas, or both. These results are consistent with the hypothesis that growth is greater for supply than for demand organs during the early period post-hatching in chickens (Lilja, 1983; Katanbaf *et al*., 1988) and turkeys (Sell *et al*., 1991). Nitsan *et al*. (1991a,b) suggested that the highest food conversion efficiency was observed during the first 10 days of age, when the relative growth reached its peak, and after the relative weights of the pancreas and small intestine are maximal. Meanwhile, a lack of the pancreatic enzyme activity decreases the apparent digestibility of the dietary components and reduces growth (Corring & Ourdon, 1977).

The results of the current study revealed that all the pancreatic enzymes were higher in CBC than MVC in the relative bases, except for amylase where there was no difference between the breeds after 10 days of age. According to Dunnington and Siegel (1995), the relative trypsin was an exception to this fact when compared between the heavy and light breeds. However, their experiment was limited to only 4 weeks. O'Sullivan *et al*. (1992) reported that the enzyme activity levels in the pancreas increase with age for relative amylase, total trypsin, total and relative chymotrypsin for the heavy breed at 3 weeks of age. Krogdahl and Sell (1989) reported that the activity of pancreatic amylase increases rapidly at the first 10 day after hatching while the trypsin increases after that.

It was obvious from the results of the current study that the absolute activities of the intestinal enzymes were greater in CBC than MVC. However, this relationship is reversed in relative bases, except for 1-d after hatching. This is due to lower enzyme secretion of MVC on one hand, and on the other hand, the effects of rapid body growth rate for CBC. These facts seemed consistent with the previous report for amylase by Leslie *et al*. (2007) who made comparisons of the high-and low-weight lines of chicks. However, their experiment was for 3 weeks of age. Furthermore, the current result agreed with the report of Cherry *et al*. (1987) concerning the intestinal trypsin and chymotrypsin activities in heavy and light breed at 61 day age. However, Dunnington and Siegel (1995) reported that among the intestinal enzymes, only the trypsin activity decreased for the heavy line compared with the light lines.

In this study, all intestinal enzyme activities in the two strains showed greater value for the jejunum rather than duodenum or ileum. These findings were expected because the pancreatic juices are emptied into the distal end of the loop of the duodenum in fowl. Thus, all the duodenal enzymes were lower than in the jejunum, and it decreased after that when reaching the

ileum in low concentration. Osman (1982) reported that the level of amylase activity was found to be high in chickens and was present in all parts of the small intestine but mainly confined to the jejunum luminal contents, from which it is assumed that the jejunum is the major site of starch digestion in chickens.

From the pattern distribution of the pancreatic and intestinal enzyme activities in this experiment, the authors did not find any correlation between the reduction of the enzyme activities in the intestinal contents and any accumulation of these enzymes in the pancreatic tissue, as reported by Pinchasov and Nitsan (1990) who suggested that the synthesis of pancreatic enzymes is regulated by the presence of chyme in the small intestine. However, O'Sullivan *et al*. (1992) showed that the levels of digestive enzymes in organs and the contents of gastrointestinal tract are influenced by genetic stock.

Both the strains in this study showed differences in their enzyme activities. When the activities were corrected for the body weight, the data failed to follow the rapid gain of the body weight for CBC, except for the 1 day post hatch. However, it was dominantly greater than MVC for the pancreatic enzymes only, whereas for CBC, the activities of the intestinal enzymes showed lower values than MVC. A comparison between these two different strains showed that genetic variation was an important source of differences, not only in body weight but also in enzymatic activities.

REFERENCES

Azahan, E. E. A., & Zahari, W. M. (1983). Observation on some characteristics of carcass and meat of Malaysian kampong chickens. *Mardi Research Bulletin, 11*, 225-232.

Cherry, J. A., Nir, I., Jones, D. E., Dunnington, E. A., Nitsan, Z., & Siegel, P. B. (1987). Growth-Associated traits in parental and F_1 populations of chickens under different feeding programs. 1. Ad Libitum feeding. *Poultry Science*, *66*, 1-9.

Corring, T., & Ourdon, D. (1977). Exclusion of pancreatic exocrine secretion from intestine in the pig; existence of a digestive compensation. *Journal of Nutrition, 107*, 1216-1221.

Dunnington, E. A., & Siegel, P. B. (1995). Enzyme activity and organ development in newly hatched chicks selected for high or low eight-week body weight. *Poultry Science*, *74,* 761-770.

Gertler, A., & Nitsan, Z. (1970). The effect of trypsin inhibitors on pancreatopeptidase E, trypsin, chymotrypsin and amylase in the pancreas and intestinal tract of chicks receiving raw and heated soya-bean diets. *British Journal of Nutrition*, *24,* 893-904.

Gyles, N. R. (1989). Poultry, people, and progress. *Poultry Science, 68,* 1-8.

Howard, F., & Yudkin, J. (1963). Effect of dietary change upon the amylase and trypsin activities of the rat pancreas. *British Journal of Nutrition, 17*, 281-294.

Katanbaf, M. N., Dunnington, E. A., & Siegel, P. B. (1988). Allomorphic relationships from hatching to 56 days in parental lines and F_1 crosses of chickens selected 27 generations for high or low body weight. *Growth Development and Aging*, *52,* 11-22.

Krogdahl, A., & Sell, J. L. (1989). Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. *Poultry Science, 68,* 1561-1568.

Leslie, M. A., Moran, E. T., & Bedford, M. R. (2007). The effect of phytase and glucanase on the ileal digestible energy of corn and soybean meal fed to broilers. *Poultry Science, 86,* 2350-2357.

Lilja, C. (1983). A comparative study of postnatal growth and organ development in some species of birds. *Growth*, *47*, 317-339.

Mitchell, M. A., & Smith, M. W. (1990). Jejunal alanine uptake and structural adaptation in response to genetic selection for growth rate in the domestic fowl (*Gallus domesticus*). *Journal of Physiology*, *424*, 7-15.

Mitchell, M. A., & Smith, M. W. (1991). The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the Domestic fowl (*Gallus Domesticus*). *Comparative Biochemistry and Physiology*, *99A*, 251-258.

Nitsan, Z., Ben-Avzaham, G., Zoref, Z., & Nir, I. (1991a). Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *British Poultry Science*, *32,* 515-523.

Nitsan, Z., Dunnington, E. A., & Siegel, P. B. (1991b). Organ growth and digestive enzyme levels to 15 days of age in lines of chickens differing in body weight. *Poultry Science, 70,* 2040-2048.

Osman, A. M. (1982). Amylase in chicken intestine and pancreas. *Comparative Biochemistry and Physiology, 73*, 751-574.

O'Sullivan, N. P., Dunnington, E. A., Larsen, A. S., & Siegel, P. B. (1992). Correlated responses in lines of chickens divergently selected for fifty-six days body weight. 3. Digestive enzymes. *Poultry Science, 71*, 610-617.

Pinchasov, Y., & Nitsan, N. Z. (1990). Metabolic and anatomical adaptations of heavy-bodied chicks to intermittent feeding. 2. pancreatic digestive enzymes. *British Poultry Science*, *31*, 769-777**.**

Pubols, M. H. (1990). Isolation, purification, and the amino acid sequence of a secretory trypsin inhibitor from the chicken pancreas. *Poultry Science, 69,* 640-646.

Pubols, M. H. (1991). Ratio of digestive enzymes in chick pancreas. *Poultry Science, 70*, 337-342.

Schreiweis, M. A., Hester, P. Y., & Moody, D. E. (2005). Identification of quantitative trait loci associated with bone traits and body weight in an F_2 resource population of chickens. *Genetics Selection Evolution,* 37, 677–698.

Sell, J. L., Angel, C. R., Piquer, F. J., Mallarino, E. G., & Al-Batshan, H. A. (1991). Development patterns of selected characteristics of the gastrointestinal tracts of young turkey poults. *Poultry Science, 70,* 1200-1205.

Pertanika J. Trop. Agric. Sci. 37 (2): 215 - 221 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

The Responses by Gut-Associated and Bronchus-Associated Lymphoid Tissues of Buffalo Calves Following Oral Exposure to *Pasteurella multocida* B:2

M. S. Abu Bakar[1], Mohd Zamri Saad[1]*, S. Jasni[1] and Zuki Abu Bakar[2]

[1]Research Centre for Ruminant Diseases, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

This report describes the mucosal immune response in the gastro-intestinal and respiratory tracts of buffalo calves following oral exposure to live wild-type *Pasteurella multocida* B:2. Nine buffalo calves of approximately 8 months old were treated with intramuscular injections of dexamethasone for 3 consecutive days before they were divided into 3 groups. Calves of group 1 were exposed orally to 50 ml inoculums containing 10^9 colony forming units (CFU)/ml of live wild-type *P. multocida* B:2. Calves of group 2 were exposed intra-trachea with 5ml of the same inocula while calves of group 3 were given 50ml of PBS orally. At the end of day 7 post-exposure, all surviving calves were killed and organs of gastro-intestinal and respiratory tracts were processed for histology examination. The presence of lymphoid nodules, the size of the nodules and the number of lymphocytes were noted. Both oral and intra-trachea exposures elicited mucosal responses in both gastro-intestinal and respiratory tracts. Oral exposure stimulated significantly ($p<0.05$) superior mucosal response in the gastrointestinal tract, while intratracheal exposure stimulated significantly ($p<0.05$) superior mucosal response in the respiratory tract. Overall, oral exposure was able to stimulate the distance mucosal sites such as the respiratory tract and provides potential use for oral administration of live vaccine against haemorrhagic septicaemia.

Keywords: Mucosal immunity, oral administration, gastro-intestinal tract, respiratory tract, *Pasteurella multocida* B:2, buffalo calves

ARTICLE INFO

Article history:
Received: 4 June 2012
Accepted: 30 July 2013

E-mail addresses:
msabuus@yahoo.com (M. S. Abu Bakar),
mzamri@upm.edu.my (Mohd Zamri Saad),
jasni@umk.edu.my (S. Jasni),
zuki@upm.edu.my (Zuki Abu Bakar)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Mucosal immune system is a critical component of animals and human defense against pathogenic organisms, especially organisms that use the mucosal surfaces as portal of entry. The mucosal membranes mediate an interface between the body and environment, which presents a variety of innate and adaptive immune defense mechanisms against microorganisms (Holmgren, 1991; Bowersock *et al.,* 1999; Gerdts *et al.,* 2001). *Pasteurella multocida* B:2 enters the hosts through the respiratory and/or oral routes leading to septicaemia (Rhoades & Rimler, 1991; Lee *et al.*, 2000). In the process of entering, *P. multocida* B:2 was found to stimulate the mucosal associated lymphoid tissue (MALT) (Siti-Raudah *et al*., 2005). Similarly, oral administered antigens have shown to elicit mucosal immune response in distant sites such respiratory, reproductive and urinary tracts (Bowersock *et al.,* 1999). This report describes the mucosal lymphoid tissue response in the gastrointestinal and respiratory tracts following oral administration of buffalo calves with live wild-type *P. multocida* B:2.

MATERIALS AND METHODS

Nine clinically healthy local buffalo calves of approximately 8 months of age were used in this study. The calves were de-wormed subcutaneously with ivomectin® (0.2mg/kg body weight) for three consecutive days, while nasal swabs were collected from all the calves at the time of arrival and then at weekly interval to ensure that they were free of *P. multocida* (Townsend *et al.,* 1998).

Meanwhile, stock culture of *P. multocida* serotype B:2 isolated from a bovine case of haemorrhagic septicaemia (HS) was used to prepare the inocula (Zamri-Saad *et al.,* 2006) of 10^9 colony forming unit (cfu)/ml (Alcamo*,* 1997). At the start of the experiment, the buffalo calves were further subdivided into three groups. All the calves were kept in individual pens but calves of groups 1 and 2 were kept in the same vicinity, while group 3 was kept separated. Calves of group 1 were exposed orally to 50ml of the inoculums while calves of group 2 were exposed intra-trachea to 5ml of the same inoculums. Calves of group 3 were the sham group that was exposed orally to 50ml of sterile PBS.

The calves were observed for adverse response or exaggerated clinical syndrome. Calves that showed severe clinical disease were euthanized; otherwise, the experiment was terminated on day 7 post-infection in accordance with the Guidelines for Animal Care and Use Committee, Universiti Putra Malaysia [AUP12R148]. During post-mortem examination, tissue samples from the nasal mucosa, bronchus and lungs of the respiratory tract, and tissues of oesophagus, rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, colon, caecum and rectum of the gastrointestinal tract were collected and placed in 10% neutral buffered formalin for at least 12 h, embedded in paraffin, sectioned at 4µm, stained with hematoxylin and eosin [HE].

Attempts were made to identify the gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT) in at least 5 microscopic fields before the sizes of BALT and GALT were determined by measuring the diameters. The numbers of lymphocytes were determined by counting the cells using the NIS element imaging software version 2.33. Data were exported to excel and subsequent predictive analysis software (PASW) for analysis.

The mean numbers of lymphocyte and the length of the diameter of the lymphatic nodule between the orally exposed, the intra-tracheal exposed and the sham-dosed calves were compared using One-ANOVA and statistical significance was considered when $p<0.05$. All the analyses were done using PASW 17.

RESULTS

Bronchus-associated Lymphoid Tissue

Both oral and intra-trachea exposed calves of groups 1 and 2 showed the presence of BALT in the respiratory tract but the calves of group 1 did not have the lymphoid aggregate in the nasal mucosa (Table 1). The calves of group 3 had only few lymphocytes found scattered in the lung parenchyma. In general, the size of lymphoid nodules and number of lymphocytes of calves of group 2 was significantly ($p<0.05$) larger than those of groups 1 and 3, while group 1 was significantly ($p<0.05$) larger than group 3 (Tables 1 and 2).

Gut-associated Lymphoid Tissue

The calves of group 1 showed significantly ($p<0.05$) larger size of lymphoid nodules in reticulum, abomasums, duodenum, jejunum, ileum and rectum (Fig.1) when compared to

TABLE 1
Mean size (µm per area) of lymphatic nodule in the respiratory tract of buffalo calves exposed to live wild-type *Pasteurella multocida* B: 2

Organ	Oral	Intra-trachea	Oral Sham-dose
Nasal mucosa	$0.00\pm0.00^{a,b}$	300.01 ± 0.59^{a}	$0.00\pm0.00^{a,b}$
Bronchus	219.00 ± 1.00^{a}	515.38 ± 0.66^{a}	0.00 ± 0.00^{a}
Lung	136.28 ± 1.00^{a}	608.44 ± 1.07^{a}	0.00 ± 0.00^{a}

Values with different superscript in the same row signifies significant difference $p<0.05$

TABLE 2
Mean lymphocyte counts (per unit area) in the bronchus-associated lymphoid tissue (BALT) of buffalo calves experimentally exposed to live wild-type *Pasteurella multocida* B:2

Organ	Oral	Intra-trachea	Oral Sham-dose
Nasal mucosa	45.0 ± 1.00^{a}	106.0 ± 1.00^{a}	8.3 ± 1.51^{a}
Bronchus	256.0 ± 3.05^{a}	425.3 ± 0.26^{a}	28.6 ± 0.84^{a}
Lung	163.7 ± 0.89^{a}	359.3 ± 1.13^{a}	7.4 ± 0.56^{a}

Values with different superscript in the same row signifies significant difference $p<0.05$

the calves of groups 2 and 3 (see Table 3). When present, the sizes of lymphoid nodules in the duodenum and jejunum of calves of group 2 were significantly ($p<0.05$) bigger than those of group 3. Similarly, the numbers of lymphocyte in the reticulum, abomasums, duodenum, jejunum, ileum and rectum were significantly ($p<0.05$) more in the calves of group 1 compared to those of groups 2 and 3 (Table 4). The numbers of lymphocytes in the reticulum, abomasums and colon of calves of group 2 were significantly ($p<0.05$) more than the numbers in calves of group 3.

DISCUSSION

This study on the response of mucosal immunity of the respiratory and gastro-intestinal tracts was based on the presence and size of lymphoid nodules and the number of lymphocytes presence in those tracts. The lymphoid nodules have been described in

TABLE 3
Mean size of the lymphoid nodule (μm per area) along the gastro-intestinal tract of buffalo calves exposed to live wild-type *Pasteurella multocida* B:2

Organ	Oral	Intra-trachea	Oral Sham-dose
Oesophagus	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Rumen	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Reticulum	71.97±1.09[a]	0.00±0.00[a,b]	0.00±0.00[a,b]
Omasum	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Abomasum	230.00±2.64[a]	0.00±0.00[a,b]	0.00±0.00[a,b]
Duodenum	301.58±1.38[a]	89.95±0.14[a]	48.65±0.91a
Jejunum	442.61±0.51[a]	310.80±0.72[a]	0.00±0.00a
Ileum	899.92±5.63[a]	165.72±0.43[a]	314.88±0.24[a]
Colon	249.83±0.32[a]	233.37±0.54[a]	239.85±1.20[a]
Rectum	659.29±0.25[a]	0.00±0.00[a,b]	0.00±0.00[a,b]

Values with different superscript in the same row signifies significant difference $p<0.05$

TABLE 4
The mean number of lymphocytes in the lymphoid nodules (per unit area) of the gastrointestinal tracts of buffalo calves exposed to live wild-type *Pasteurella multocida* B:2

Organ	Oral	Intra-trachea	Oral Sham-dose
Oesophagus	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Rumen	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Reticulum	262.3±1.04[a]	42.3±0.30[a]	0.00±0.00[a]
Omasum	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Abomasum	436.0±2.00[a]	24.3±0.88[a]	0.00±0.00[a]
Duodenum	233.3±0.30[a]	0.00±0.00[a]	59.5±0.00[a]
Jejunum	259.3±1.13[a]	229.3±0.70[a]	101.2±2.54[a]
Ileum	474.3±1.47[a]	126.3±1.04[a,b]	126.3±3.25[a,b]
Colon	353.3±1.70[a]	346.3±1.13[a]	0.00±0.00[a]
Rectum	652.7±3.35[a]	0.00±0.00[a]	93.0±1.41[a]

Values with different superscript in the same row signifies significant difference $p<0.05$

the respiratory tract and gastro-intestinal tracts of calves (Saw *et al*., 2004; 2005), while the diffuse lymphoid tissue, solitary lymphocytes, intraepithelial lymphocytes, lymphoid nodule and Peyer's patches have been used as tools for assessment of mucosal immune response (Shewen *et al.,* 2009). Following oral and intra-trachea exposure of calves to live *P. multocida* B:2, both GALT and BALT were stimulated in the size and number of cells compared to the non-exposed calves. Needless to say, those exposed orally showed significantly better response by GALT while those exposed intra-trachea showed significantly better response by the respiratory tract. This finding re-emphases and confirms the previous reports that concluded the most effective way of inducing mucosal immunity is the delivery of antigen at the portal of entry of the microorganism (Bowersock *et al.,* 1999). In contrast to the speculations of problematic nature of ruminants gastro-intestinal mucosa and possibility of microbial degradation by the rumen (Shewen *et al.,* 2009), oral administration of live *P. multocida* B:2 elicited more diffuse lymphatic tissue count and wider lymphatic nodular diameter at the point of delivery as well as at the distant sites, as observed earlier following the intra-tracheal administration of *P. multocida* B:2 (Saw *et al*., 2005). This is evident by comparable lymphatic nodules in the respiratory tract and wider organ coverage in gastrointestinal tract.

Nevertheless, the responses by mucosal immunity of both gastro-intestinal and

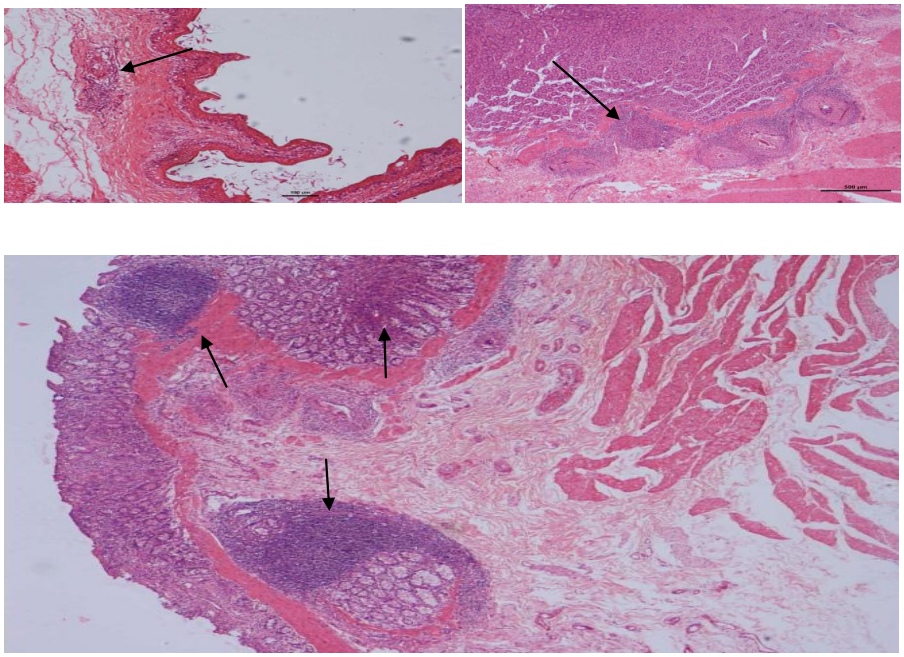


Fig.1: The gut-associated lymphopid tissue (arrows) observed in the reticulum (above, left), jejunum (above, right) and rectum of buffalo calves exposed orally to live wild-type *Pasteurella multocida* B:2

respiratory tracts were significant and in agreement to the earlier reports that there is a common mucosal pathway which enables administration of antigen at a mucosal site to stimulate mucosal immune response in the distant mucosal sites (Bowersock *et al.,* 1999). Therefore, further study should focus on stimulating mucosal associated lymphoid tissue using orally administered vaccine or antigen and assess the protective capacity provided by such vaccination programme in the control strategy of haemorrhagic septicaemia.

REFERENCES

Alcamo, I. E. (1997). *Fundamental of Microbiology* (5th edn). Menlo Park, California: Addison Wesley Longman.

Bowersock, T. L., HogenEsch, H., Suckow, M., Guimond, P., Martin, S., Borie, D., Torregrosa, S., Park, H., & Park, K. (1999). Oral vaccination of animals with antigens encapsulated in alginate microsphers. *Vaccine, 17,* 1804-1811.

Eldridge, J., Hammond, C., Meulbroek, J., Staas, J. A., Gilley, R. M., & Tice, T. R. (1990). Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. *Journal of Controlled Release, 11,* 205-211.

Gerdts, V., Uwiera, R. R. E., Mutwiri, G. K., Wilson, D. J., Bowersock, T., Kidane, A., Babiuk, L. A., & Griebel, P. J. (2001). Multiple intestinal 'loops' provide an in vivo model to analyse multiple mucosal immune responses. *Journal of Immunological Methods, 256,* 19-33.

Holmgren, J. (1991). Mucosal immunity and vaccination, *FEMS Microbiology Immunology, 89,* 1-10.

Lee, C. W., Wilkie, I. W., Townsend, K. M., & Frost, A. J. (2000). The demonstration of *Pasteurella multocida* in the alimentary tract of chickens after experimental oral infection. *Veterinary Microbiology, 72,* 47-55.

Saw Po Po, Zuki, A. B. Z., Zamri-Saad, M., Omar, A.R., & Effendy, A. W. M. (2004). Distribution and morphological study of the bronchus associated lymphoid tissues (BALT) in three months old calves. *Journal of Animal and Veterinary Advances, 3,* 564-570.

Saw, Po Po, Zuki, A. B. Z., Zamri-Saad, M., Omar, A. R., & Effendy, A. W. M. (2005). Morphological study of the jejunal and ileal peyer"s patches of three-month old calves. *Journal of Animal and Veterinary Advances, 4,* 579-589.

Rhoades, K. R., & Rimler, R. B. (1991). Fowl cholera. In B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, & H. W. Yoder, Jr. (Eds). *Diseases of Poultry* (pp. 145-162). Iowa State University Press, Ames, IO, USA.

Shewen, P. E., Carrasco-Medina, L., McBey, B. A., & Hodgins, D. C. (2009). Challenges in mucosal vaccination of cattle. *Veterinary Immunology and Immunopathology, 128,* 192-198.

Siti-Raudah, S. A. K., Effendy, A. W. M., Zamri-Saad, M., & Zuki, M. B. Z. (2005). Response of bronchus- associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT) following administration of formalin-killed *Pasteurella multocida* B:2. Proceeding of the Regional Symposium on Haemorrhagic Septicaemia, Palm Garden Hotel, Putrajaya, Malaysia 1st -2nd December, pp. 45-49.

Townsend, K. M., Frost, A. J., Lee, C. W., Papadimitrious, J. M., & Dawkins, H. J. S. (1998). Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. *Journal of Clinical Microbiology, 36,* 1096-1100.

Zamri-Saad, M., Ernie, A., & Sabri, M. Y. (2006). Protective effect following intranasal exposure of goats to live *Pasteurella multocida* B:2. *Tropical Animal Health and Production, 38,* 541-548.

Pertanika J. Trop. Agric. Sci. 37 (2): 223 - 247 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Increasing Rice Production Using Different Lime Sources on an Acid Sulphate Soil in Merbok, Malaysia

Elisa Azura Azman, Shamshuddin Jusop*, Che Fauziah Ishak and Roslan Ismail

Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Acidity is released in high amounts when pyrite-bearing sediments in the coastal plains of Malaysia are drained for development, either agriculture or otherwise. The soils formed from these materials are called acid sulphate soils, which are characterized by low pH and high exchangeable Al that adversely affect plant growth. A study was conducted with the objective of increasing rice yields on these soils under rain-fed condition in Merbok, Kedah, Malaysia, using various lime sources. The acid sulphate soil was treated with ground magnesium limestone (GML), hydrated lime and liquid lime at specified rates. Paddy variety MR 219 was tested in a field experiment as this variety is the most common variety grown in Malaysia. Prior to treatments, the pH of water sample in the rice field was 3.7, while Al concentration was 878 µM. Thus, rice plants grown under these conditions would suffer from H^+ and Al^{3+} stress without amelioration, thus retard and/or minimize rice growth and yield. In the first season (1st season) rice plants were affected by drought during the vegetative period, while in the subsequent season (2nd season), they were infested with rice blast fungus (*Magnaporthe grisea*). In spite of that, however, the rice yield was 3.5 t ha^{-1} based on the application of 4 t GML ha^{-1}, which was almost equivalent to the average national yield of 3.8 t ha^{-1}. As a result, it was noted that the ameliorative effects of lime application in the 1st season had continued to the 2nd season. Liming at 4 t GML ha^{-1} incurs high cost to the farmers. However, the yield obtained is worth the effort and cost.

ARTICLE INFO

Article history:
Received: 9 January 2013
Accepted: 9 September 2013

Keywords: Acid sulphate soil, aluminium, ground magnesium limestone, pyrite, rice, rice blast

E-mail addresses:
elisa1814@gmail.com (Elisa Azura Azman),
shamshud@upm.edu.my (Shamshuddin Jusop),
cfauziah@upm.edu.my (Che Fauziah Ishak),
roslanismail@upm.edu.my (Roslan Ismail)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Global demand for rice is increasing by the years. This means that the world needs to produce more rice than it does now, and this is part of the agenda in food security that has been addressed in the World Food Summit 1996. However, in many areas with high population density, highly productive rice land has been lost to housing and industrial development and/or to growing of vegetables and other cash crops. Plus, the possibility of increasing area for rice cultivation is almost nil, and this is mainly because arable land has been exhausted in most Asian countries. Arable lands are marked by good and fertile land for agriculture production.

Rice is a staple food for Malaysians. Therefore, the government of Malaysia realizes that it needs to increase self-sufficiency level (SSL) in rice production from 73% to 86%. In order to increase SSL, there are three possible alternatives: 1) expanding the rice cultivation area, 2) increasing the yield per unit area, and/or 3) combination of alternatives 1 and 2. At present condition, with scarcity of good and fertile lands, minimal expansion in rice area can be expected, coupled with slow increase in rice yield. In reality, growth in rice production is in contrast to demand. For that reason, farmers need to increase their rice production on land that is previously idle and less fertile such as the acid sulphate soils in Malaysia. These soils have low pH and high Al content which can be detrimental for crop production. Expanding rice-growing areas in such a challenging area must be done with great care. Rice cultivation must be sustainable with minimal environmental impact on the ecosystem.

Acid sulphate soils are widespread in Malaysia, occurring almost exclusively along its coastal plains (Shamshuddin & Auxtero, 1991; Shamshuddin *et al*., 1995; Muhrizal *et al*., 2006; Enio *et al*., 2011). These soils are dominated by pyrite (FeS_2) and marked with high acidity (soil pH< 3.5). These soils are produced when the pyrite-laden soils in the coastal plains are opened up for crop production and/or development. This scenario leads to release of high amounts of Al into the soil environment (Shamshuddin *et al*., 2004b) and affects crop growth. As an example, it affects oil palm growth (Auxtero & Shamshuddin, 1991) and cocoa production (Shamshuddin *et al*., 2004a), but kills plants and aquatic life in the surrounding areas. Despite the abovementioned limitations, about 3000 ha of land in Merbok, Kedah, have been cultivated with rice since 1964 (Ting *et al*., 1993), but the yield is far below the national average of 3.8 t ha^{-1}.

Among the major agronomic problems common to acid sulphate soils are toxicity due to the presence of Al, decrease of P availability, nutrient deficiencies, and Fe (II) toxicity (Dent, 1986; Elisa *et al*., 2011). Thus, under normal circumstances, acid sulphate soils are not suitable for crop production, unless some amelioration practices are made. Among the practices are liming with ground magnesium limestone (GML), submergence, leaching, applying manganese dioxide (Park & Kim, 1970), phosphate application and applying basalt.

From all of the above practices, liming is the common approach to raise pH. By increasing soil pH to more than 5, soluble Al often precipitates in soil as gibbsite ($Al(OH)_3$), thereby reduces Al toxicity in soil. Besides increasing soil pH, GML can supply large quantity of Ca and Mg for crop uptake, which is essential nutrient for good rice growth. Furthermore, Ting *et al*. (1993) stated that rice yield increased from < 2 to 4.5 t ha^{-1} seasons after annual GML application of 2 t ha^{-1}.

Besides liming material, organic fertilizers can also be applied to acid sulphate soils. Under flooded condition, these organic fertilizers supply NPK and alleviate Al toxicity in the acid sulphate soils (Muhrizal *et al*., 2003). Meanwhile, in another study under flooded, reduced and re-flooded conditions, organic materials (acting as organic fertilizers) in combination of Fe (III) oxides does not increase soil pH above 5 (Muhrizal *et al*., 2006). This means that, to some extent, the Al is still present in the solution at toxic level.

On the other hand, Suswanto *et al*. (2007) found that under field trial condition, application of GML+organic fertilizer can produce rice yield up to 7.5 t ha^{-1} (Suswanto *et al*., 2007). Therefore, with applications of lime, basalt, organic fertilizer and/or their combinations at appropriate rates, acid sulphate soils are able to be ameliorated (Suswanto *et al*., 2007; Shazana *et al*., 2011). The current study was conducted to determine the effects applying lime from various sources for rice production on an acid sulphate soil under rain-fed condition in Merbok, Kedah, Malaysia.

MATERIALS AND METHODS

Background of the Study Area

This study was conducted in Merbok, Kedah, and the soil is an acid sulphate soil (Merbok series). At the study site, approximately 3000 ha are being utilized for rice cultivation for more than 40 years using fertilizers and pesticides subsidized by the Malaysian government. This area has been experiencing low rice yield with an average production of less than 2 t ha^{-1} $season^{-1}$. Besides that, this area is often exposed to severe infection of *Magnaporthe grisea* fungal disease, more commonly known as rice blast, which further reduces yield. To make matters worse, the farmers rely solely on rain water (rain-fed condition) as there is no irrigation system in this area. Formerly, these areas were occupied by high tidal mangrove flats and were converted to paddy fields in 1964. The mean rainfall recorded at these areas is 2155 mm $year^{-1}$, with pronounced dry period in December-March annually. During these dry periods, temperature reaches 50°C thus evapo-transpiration rate exceeds precipitation as described by Ting *et al*. (1993).

Soil and Site Description

Field trials were conducted in Merbok, Kedah, Malaysia (5.7185 N, 100.3812 E) (Fig.1). The experimental plots were established on an acid sulphate soil classified as Merbok Series (Paramananthan, 1987) which is Typic Sulfaquents (Soil Survey Staff, 2010). This area has been cultivated with paddy for more than 40 years by farmers using fertilizers and pesticides

subsidized by the Malaysian government. This area has been experiencing low rice yield, with an average production of < 2 t ha^{-1} $season^{-1}$. It is often exposed to severe infection of rice blast which further reduces yield. At the onset of the current experiment (March 2010), soils were sampled at 15 cm interval to the depth of 75 cm at selected locations in the experimental plots in order to determine their original chemical properties (Table 1). The texture is clay loam with 31.25% sand, 39.36% silt and 29.18% clay. The topsoil (0–15 cm depth) contains 2.78% total carbon, 0.19% total N, 2.28 mg kg^{-1} available P, 0.31 $cmol_c$ kg^{-1} exchangeable K and 6.19 $cmol_c$ kg^{-1} exchangeable Al. Soil pH is 3.4.

Experimental Design, Treatments and Field Management

In this study, Randomized Completely Block Design (RCBD) was used with five treatments replicated five times. The plot size was 5.0 m x 5.0 m and the plots were separated from one another by sealed ridge (sealed using plastic film; the depth was 15 cm under the soil surface) to prevent water movement among the plots.

The soils were treated with GML, hydrated lime or liquid lime at the rate shown in Table 2. GML and hydrated lime were applied only once during the 1st season (dry season), a month prior to sowing. These liming materials were evenly distributed and incorporated within the topsoil. For liquid lime treatment, 20 L ha^{-1} was mixed with water at ratio of 1:5 and sprayed onto the soil surface a day before sowing.

TABLE 1
Initial chemical characteristics of the soil at various depths prior to sowing

Depth (cm)	pH water (1:2.5)	EC (dS m^{-1})	Exchangeable cations (cmolc kg^{-1})					Fe (mg kg^{-1})	CEC (cmolc kg^{-1})	Total N (%)	Total carbon (%)	Available P (mg kg^{-1})
			K	Ca	Mg	Na	Al					
0-15	3.40	0.78	0.25	2.37	2.56	0.12	6.19	525.00	10.36	0.19	2.78	2.28
15-30	2.36	1.08	0.21	2.42	2.80	0.29	7.82	284.70	10.71	0.10	1.82	1.53
30-45	2.90	1.73	0.91	2.57	2.99	0.44	8.53	316.40	11.93	0.10	1.89	1.44
45-60	2.93	2.17	0.22	2.53	3.65	0.69	8.63	307.50	13.21	0.10	2.30	1.58
60-75	2.81	4.06	0.23	2.85	4.63	1.44	10.02	560.55	17.64	0.12	3.54	2.11

Rice (*Oryza sativa*) variety MR 219 with 90% germination rate was used. This is the rice variety that is commonly planted by the farmers throughout Peninsular Malaysia. Seeds were sown during April 2010 and October 2010 for the first and second season, respectively, at a seeding rate of 150 kg ha^{-1}. The seeds were soaked with hormone-based chemical (Zappa™) for 24 hours. The seeds were rinsed with tap water and left in the dark place for 24 hours before sowing in the field.

Fertilizers were applied in the experimental plots based on standard fertilizer rate (120 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1}, 80 kg K_2O ha^{-1}) using urea, NPK Blue (12:12:17+TE) and NPK Green (15:15:15+TE) as the sources of the nutrients. Growth enhancers, namely Vita-grow™ and Robust™, were applied 15, 45

TABLE 2
Treatments in the field

Symbol	Treatments
T1	Control (without lime)
T2	4 t ha^{-1} ground magnesium limestone (GML)
T3	2 t ha^{-1} hydrated lime
T4	20 L ha^{-1} of liquid lime (only apply for the 1st season)
T5	20 L ha^{-1} of liquid lime (apply for 1st and 2nd season)

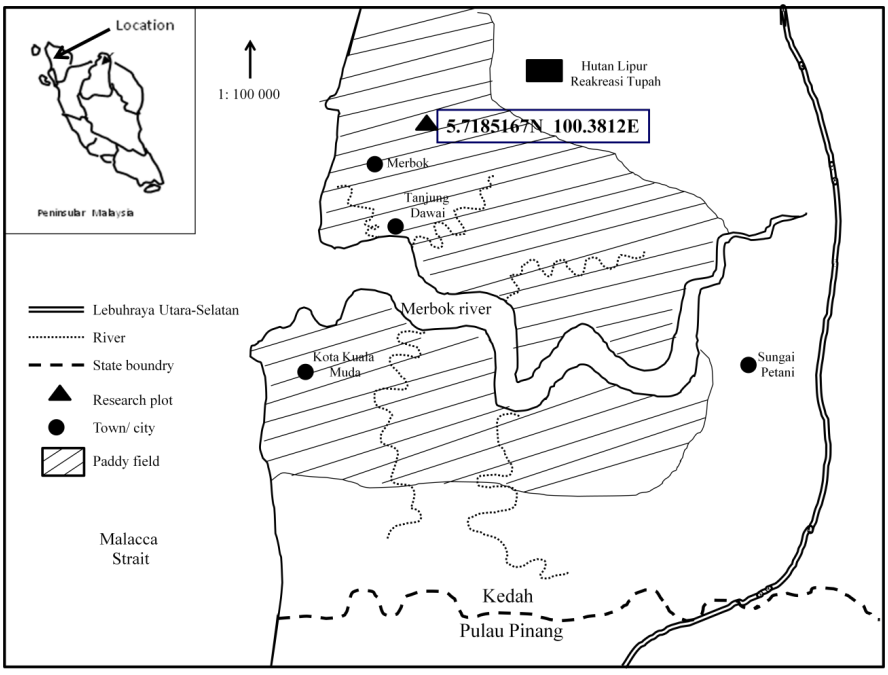


Fig.1: Map indicating Merbok in Kedah, where the field trial was carried out

and 60 days after seeding (DAS) at the rate of 75 mL and 100 mL, respectively. Both growth enhancers were mixed with 20 L of water for 1 ha of paddy field to boost the growth.

During the first season (April-August, 2010), there was an extended dry period during the vegetative and reproductive phases. Therefore, water needed to be pumped from the nearest drainage canal (acidic water) to ensure that the rice seeds were germinated. On the other hand, there was no water limitation during the second season (September 2010-January 2011) due to intermittent heavy rainfall throughout the season. The crop of rice was harvested in August 2010 and January 2011 for the first and second seasons, respectively.

Soil Sampling and Chemical Analysis

Soil sampling was carried out three times: (i) before rice planting of the first season (April 2010); (ii) after the first harvest (August 2010); and (iii) after second harvest (February 2011). Only topsoil (0-15 cm) was sampled and three samples were taken from each experimental plot using a soil auger. After air-drying, the soil samples were ground and passed through a 10-mesh sieve (2 mm). The following soil analyses were carried out: (i) Soil pH was determined in water at soil to solution ratio of 1:2.5; (ii) cation exchange capacity (CEC) was determined by 1 M NH_4OAc at pH 7 (Chapman, 1965); (iii) exchangeable Ca, Mg and K in the NH_4OAc extract were determined by Perkin Elmer Analyst 400 atomic absorption spectrometry (AAS); (iv) determination of exchangeable Al was done using 5 g of air-dried soil, extracted with 50 mL of 1 M KCl. The mixture was shaken for 30 minutes and filtered using a filter paper (Whatman No. 42) before determining the Al by AAS; and (v) extractable Fe was determined using double acid method. Fe was extracted using 0.05 M HCl in 0.0125 M H_2SO_4. Five g of air-dried soil was mixed with 25 mL extracting solution, shaken for 15 minutes and centrifuged at 180 rpm. The supernatant was then filtered through filter paper (Whatman no 42) and the Fe was determined using AAS. The analysis methods are detailed in Carter *et al*. (1993).

Harvesting and Yield Component Measurements

The crops were harvested on 29th August, 2010 and 13th February, 2011 for the first and second seasons, respectively. During harvest, a quadrate of 25 cm x 25 cm size was used for sampling the plant parts. The quadrate was thrown 4 times randomly in each of the experimental plot. The samples were taken to the laboratory for yield components analysis.

The following yield components analysis were determined: (i) panicle number was determined by counting all the panicles from each quadrate sampling and 20 panicles were selected randomly from each experimental plot for further yield component analysis; (ii) panicle length was measured using a ruler; (iii) determination of spikelet per panicle was done by threshing the grains from the samples and unfilled grains were separated from filled grains using the

seed separator; (iv) percentage of filled spikelet was calculated using a formula (filled spikelet per panicle/total spikelet per panicle) x 100; and (v) 1000 grain weight. Grain yield was determined from all plants from a 25 m^2 site (except border plants) in each experimental plot.

Plant Tissue Analyses

The upper part of the plants was oven-dried at 65ºC for three days. The samples were ground using a stainless steel grinder and passed through a 1-mm sieve. The samples (0.25 g) were then digested by wet-ashing using 1:1 ratio H_2SO_4-H_2O_2 on a block digester at 350ºC. The digested solutions were filtered through Whatman filter paper No. 42 and made up to 100 mL volume with distilled water. The concentrations of calcium (Ca), magnesium (Mg), aluminum (Al) and iron (Fe) were measured using Perkin-Elmer AAnalyst 400 AAS. Nitrogen (N) and potassium (K) were measured using Lachat QuickChem® FIA+ 8000 Series auto analyzer (AA).

Analysis of Water from the Field Plots

Water was collected from each of the experimental plots. The samples were taken every week for the first 5 weeks, followed by every 2 weeks until harvest. For the first season, the sampling started at 14 DAS due to dry conditions on the field at 7 DAS, while for the second season, the sampling was stopped at 77 DAS when the paddy field dried up. After filtering the samples, pH was determined using Sartorius pH meter PB-11. Al and Fe concentrations were determined using Perkin-Elmer AAnalyst 400 AAS.

Statistical Analysis

Data from the experiment were analyzed statistically using analysis of variance (ANOVA), and least significant difference (LSD) test was employed to determine the mean differences between the treatments. The statistical package used was SAS v9.1 software.

RESULTS AND DISCUSSION

Changes in soil properties

The soil under investigation is low in pH and high in exchangeable Al (Table 1). Soil pH throughout the soil profile is < 3.50. This low pH is consistent with the presence of jarosite in the sub-soil, which qualifies it to be classified as an acid sulphate soil (*Typic Sulfaquents*). Exchangeable Al in the soil is very high throughout the soil depth. The topsoil (0-15 cm depth) is the zone where the development of rice root occurs. The pH values and exchangeable Al of the topsoil are 3.4 and 6.19 $cmol_c\ kg^{-1}$, respectively (Table 1). The concentration of Al exceeds the critical level for rice production of 1-2 mg kg^{-1}, as suggested by Dobermann and Fairhust (2000). The pH and the concentration of Al in the water at the soil pit is 3.70 and 878 µM, respectively. The concentration of Al is far above the critical toxic level of 74 µM for rice growth as suggested by Dent (1986). The favourable pH for optimal rice (MR 219) root growth is 6 (Elisa *et al*., 2011).

However, to raise the pH up to this level is costly and many ordinary farmers may not be able to afford it. Aluminium toxicity can occur in soil when pH < 3.5 (van Breemen & Pons, 1978). A study conducted in Japan showed that the growth of Al-tolerant rice variety began to be inhibited when the Al^{3+} ion concentration exceeded 900 µM (Cate & Sukhai, 1964). This value is close to aluminium concentration in this study at 878 µM; thus, rice growth in this study area can be inhibited by Al. Moreover, the rice variety used in the current study is not Al-tolerant.

First Season

The first season started in August 29, 2010. The result showed that treating the soil with 4 t GML ha^{-1} was able to increase rice production by 29.17% from 2.50 t ha^{-1} (control) to 3.53 t ha^{-1}, and this value was slightly higher than average rice yield using farmer's practice of less than 2 t ha^{-1} $season^{-1}$ (Table 3). However, this yield was not significantly different from the control. Meanwhile, application of 4 t GML ha^{-1} produced the highest value in terms of panicle number m^{-2}, spikelet number per panicle, 1000 grain weight and panicle length, with values of 914, 132, 25.30 g and 24.65 cm, respectively, among the other treatments. However, there was no significant difference among the treatments for panicle number m^{-2}. There were significant differences observed for the percentage of filled spikelet. The means that treating with 2 t ha^{-1} of hydrated lime was significantly higher compared to treating with 20 L ha^{-1} of liquid lime, with values of 73.13% and 61.27%, respectively. Based on LSD, there were significant differences observed for the 1000 grain weight and panicle length.

In this study, it was observed that relative rice yield was affected by the soil pH and exchangeable Ca (Fig.2). It means that as the soil pH and exchangeable Ca increase, the relative rice yield also increases. The

TABLE 3
Mean rice grain yield and its components for the first and second seasons

Seasons	Treatments	Actual yield (t ha^{-1})	Panicle number m^{-2}	Spikelet num/ panicle	Filled spikelet (%)	1000 grain weight (g)	Panicle length (cm)
S1	T1	2.50^{ab}	794^{a}	120^{ab}	68.02^{bc}	23.00^{b}	23.03^{ab}
	T2	3.53^{a}	914^{a}	132^{a}	71.23^{ab}	25.30^{a}	24.65^{a}
	T3	3.24^{a}	866^{a}	118^{ab}	73.13^{a}	24.70^{a}	24.14^{a}
	T4	1.79^{b}	763^{a}	101^{b}	64.27^{cd}	22.80^{b}	21.65^{b}
	T5	1.57^{b}	831^{a}	103^{b}	61.27^{d}	22.36^{b}	22.05^{b}
S2	T1	2.10^{a}	610^{a}	144^{a}	71.45^{a}	24.89^{a}	24.56^{a}
	T2	1.90^{a}	679^{a}	153^{a}	71.56^{a}	23.10^{a}	23.80^{a}
	T3	1.88^{a}	675^{a}	150^{a}	68.51^{a}	24.89^{a}	24.68^{a}
	T4	1.84^{a}	607^{a}	134^{a}	70.57^{a}	25.12^{a}	24.43^{a}
	T5	1.60^{a}	657^{a}	132^{a}	68.61^{a}	24.90^{a}	24.43^{a}

Means followed by the same letter within a column are not significantly different (LSD's test, P > 0.05).

relative rice yield is positively correlated with soil pH (Fig.2a) and exchangeable Ca (Fig.2b) and the corresponding relationship is given by equation Y= 91.10x – 238.36 (R^2=0.70) and Y= 49.86x + 30.30 (R^2=0.49), respectively. The pH value corresponding to 90% relative yield is 3.60. The critical exchangeable Ca is 1.197 $cmol_c\ kg^{-1}$, which is comparable to that found by Dobermann and Fairhust (2000). High Ca, to some extent, is able to reduce Al toxicity (Alva *et al*., 1986).

The yield for the first season can be increased with proper field management. Besides high soil acidity and Al toxicity, farmers in this area are facing another problem, which is drought. Bouman and Tuoang (2001) wrote that lowland rice is extremely sensitive to water shortage and drought problem when soil water contents drop below saturation and this will reduce leaf area expansion, closure of stomata, leaf rolling, deeper root growth, enhanced leaf senescence, reduced plant height, delayed flowering and reduced number of tillers, panicle, spikelet and grain weight. In the current study, the paddy field was dry when the seeds were sown during the

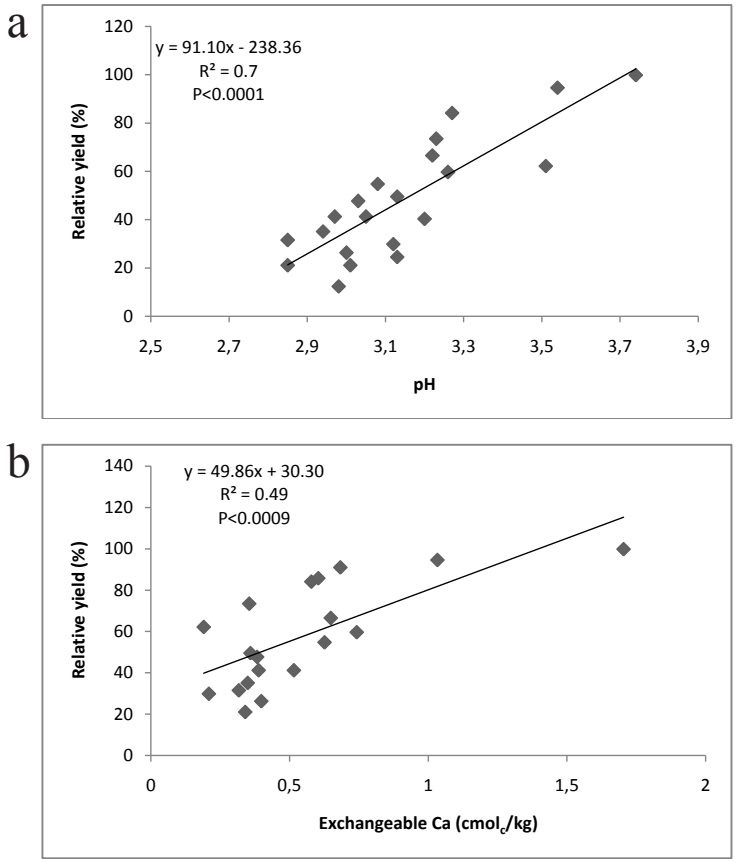


Fig.2: Relationship between: (a) relative yield and pH and (b) relative yield and exchangeable Ca for the first season

first season. There was no proper water management practice in the area where the farming communities depend solely on rain water that falls erratically throughout the growing season; hence, it was insufficient. As a result, the broadcasted seeds did not germinate well and the seedlings suffered because their roots were unable to tap the underground water. Therefore, acid water was pumped in from the nearest drainage canal to germinate the seeds. This had affected the subsequent growth of rice seedlings and hence the eventual rice yield.

The acid water contains Al concentration at 878 µM with pH of 3.70. This Al concentration is far above the critical toxic level of 74 µM for rice growth, as suggested by Dent (1986). Furthermore, Zhu *et al*. (2009) mentioned that rice is expected to suffer from H^+ stress if grown on a soil with low pH. Growing rice in an area with low pH and high Al concentration would inhibit the elongation of plant roots (Horst *et al*., 2009). There will be disruption of root cap forming processes, decline in cell division and deposition of lignin (Susan *et al*., 2007). In the end, root length is inhibited. As a result, nutrient uptake is curtailed and multiple nutrient deficiencies occur (Godbold *et al*., 1988; Tan & Keltjens, 1995; Ridolfi & Garrec, 2000), and this has been proven by this study which showed that the concentration of Ca in the root was <0.01% (Table 4) due to the presence of high Al. Elongation of root length is well associated with root surface area. Root surface area of rice seedling needs to be increased for better absorption of nutrients and this can be done by raising solution pH by using lime. At 42 days after sowing, the water level in the plot was about 30 cm due to heavy rainfall. Thus, the ripening period was delayed to 125 DAS. This had affected the time for harvesting and pest started to attack the rice, resulting in a lower yield than had otherwise been expected.

Rice is known to tolerate some levels of acidity. Table 5 shows the effects of lime

TABLE 4
Mean nutrients concentrations of the above ground parts and root at 75 day after seeding

Seasons	Treatments	Upper part (%)						Root (%)					
		N	K	Ca	Mg	Al	Fe	N	K	Ca	Mg	Al	Fe
S1	T1	2.62^{ab}	2.78^{ab}	0.14^{ab}	0.27^{b}	0.03^{b}	0.16^{a}	1.86^{a}	1.19^{a}	6.8x10$^{-4\,b}$	0.09^{ab}	1.74^{a}	4.38^{a}
	T2	2.33^{b}	2.58^{bc}	0.13^{ab}	0.33^{a}	0.06^{a}	0.15^{a}	1.56^{a}	1.02^{a}	1.9x10$^{-3\,a}$	0.10^{a}	1.77^{a}	4.71^{a}
	T3	2.36^{b}	2.43^{c}	0.14^{a}	0.28^{ab}	0.04^{ab}	0.16^{a}	1.76^{a}	1.24^{a}	7.8x10$^{-4\,b}$	0.09^{ab}	2.25^{a}	4.65^{a}
	T4	2.85^{a}	2.93^{a}	0.11^{ab}	0.27^{b}	0.04^{ab}	0.16^{a}	1.87^{a}	1.26^{a}	6.4x10$^{-4\,b}$	0.07^{b}	1.92^{a}	4.55^{a}
	T5	2.69^{ab}	2.77^{ab}	0.11^{ab}	0.26^{b}	0.04^{ab}	0.19^{a}	1.74^{a}	1.05^{a}	3.0x10$^{-4\,b}$	0.07^{b}	1.75^{a}	5.27^{a}
S2	T1	2.40^{ab}	2.28^{ab}	0.12^{a}	0.27^{a}	0.03^{a}	0.05^{a}	1.26^{a}	0.64^{ab}	1.8x10$^{-3\,b}$	0.06^{a}	1.56^{a}	3.48^{a}
	T2	2.84^{a}	2.54^{a}	0.12^{a}	0.26^{a}	0.04^{a}	0.05^{a}	1.27^{a}	0.55^{b}	4.0x10$^{-3\,a}$	0.06^{a}	1.40^{a}	3.03^{a}
	T3	2.45^{ab}	2.47^{a}	0.12^{a}	0.28^{a}	0.04^{a}	0.05^{a}	1.15^{a}	0.65^{ab}	2.8x10$^{-3\,ab}$	0.06^{a}	1.82^{a}	2.76^{a}
	T4	2.19^{ab}	2.07^{ab}	0.11^{a}	0.26^{a}	0.04^{a}	0.06^{a}	1.26^{a}	0.72^{ab}	2.0x10$^{-3\,b}$	0.05^{a}	1.73^{a}	3.10^{a}
	T5	2.39^{ab}	2.28^{ab}	0.11^{a}	0.26^{a}	0.04^{a}	0.05^{a}	1.08^{a}	0.82^{a}	3.0x10$^{-3\,ab}$	0.06^{a}	1.96^{a}	3.09^{a}

on the soil properties in the Merbok trial. It is seen that pH is still below 5 after the first harvest. According to Ponnamperuma *et al*. (1973), only at pH below 4, rice was adversely affected. Soil pH for treatment with 2 t ha^{1-} of hydrated lime was the highest with 3.36 and it is higher than treatment with 20 L ha^{-1} of liquid lime and the control. Brady (1974) mentioned that hydrated lime reacted with the soil much more rapidly than its carbonate form. However, dolomitic limestone is often preferred because it supplies significant quantity of Mg. Besides that, GML can stay reasonably longer in the soil compared with hydrated lime. Nonetheless, exchangeable Al did not show any significant difference among the treatments.

Fig.3 shows the pH, Al and Fe concentrations of water from the field with time for the first season. The water was sampled every week for the first 5 weeks, followed by every 2 weeks until harvest. However, the sampling of water was started in the second week after sowing due to dry condition (Fig.4a). Therefore, water was pumped in from the nearest drainage canal to irrigate the experimental plots (Fig.4b). It is common knowledge that GML increases soil pH. Liming is a standard agronomic practice to increase pH of acid sulphate soils and this phenomenon is clearly shown in Fig.3a. However, the application rates of liming materials are dependent on localities; hence, field experiment such as conducted in this study are often necessary to justify the most suitable and feasible application rate.

Soil pH started to increase immediately after the field plots were flooded. It reached maximal value after 4 weeks. The increase was also due to reduction process that had taken place. Fig.3b shows the Al concentration was lower with the applications of 4 t GML ha^{-1} and 2 t hydrated lime ha^{-1} compared to the control. It seemed that the pH was still low and Al concentration was still high in the water in the research plots and these explained why rice yield was not

TABLE 4
Mean nutrients concentrations of the above ground parts and root at 75 day after seeding

Seasons	Treatments	Upper part (%)						Root (%)					
		N	K	Ca	Mg	Al	Fe	N	K	Ca	Mg	Al	Fe
S1	T1	2.62^{ab}	2.78^{ab}	0.14^{ab}	0.27^{b}	0.03^{b}	0.16^{a}	1.86^{a}	1.19^{a}	6.8x10$^{-4\,b}$	0.09^{ab}	1.74^{a}	4.38^{a}
	T2	2.33^{b}	2.58^{bc}	0.13^{ab}	0.33^{a}	0.06^{a}	0.15^{a}	1.56^{a}	1.02^{a}	1.9x10$^{-3\,a}$	0.10^{a}	1.77^{a}	4.71^{a}
	T3	2.36^{b}	2.43^{c}	0.14^{a}	0.28^{ab}	0.04^{ab}	0.16^{a}	1.76^{a}	1.24^{a}	7.8x10$^{-4\,b}$	0.09^{ab}	2.25^{a}	4.65^{a}
	T4	2.85^{a}	2.93^{a}	0.11^{ab}	0.27^{b}	0.04^{ab}	0.16^{a}	1.87^{a}	1.26^{a}	6.4x10$^{-4\,b}$	0.07^{b}	1.92^{a}	4.55^{a}
	T5	2.69^{ab}	2.77^{ab}	0.11^{ab}	0.26^{b}	0.04^{ab}	0.19^{a}	1.74^{a}	1.05^{a}	3.0x10$^{-4\,b}$	0.07^{b}	1.75^{a}	5.27^{a}
S2	T1	2.40^{ab}	2.28^{ab}	0.12^{a}	0.27^{a}	0.03^{a}	0.05^{a}	1.26^{a}	0.64^{ab}	1.8x10$^{-3\,b}$	0.06^{a}	1.56^{a}	3.48^{a}
	T2	2.84^{a}	2.54^{a}	0.12^{a}	0.26^{a}	0.04^{a}	0.05^{a}	1.27^{a}	0.55^{b}	4.0x10$^{-3\,a}$	0.06^{a}	1.40^{a}	3.03^{a}
	T3	2.45^{ab}	2.47^{a}	0.12^{a}	0.28^{a}	0.04^{a}	0.05^{a}	1.15^{a}	0.65^{ab}	2.8x10$^{-3\,ab}$	0.06^{a}	1.82^{a}	2.76^{a}
	T4	2.19^{ab}	2.07^{ab}	0.11^{a}	0.26^{a}	0.04^{a}	0.06^{a}	1.26^{a}	0.72^{ab}	2.0x10$^{-3\,b}$	0.05^{a}	1.73^{a}	3.10^{a}
	T5	2.39^{ab}	2.28^{ab}	0.11^{a}	0.26^{a}	0.04^{a}	0.05^{a}	1.08^{a}	0.82^{a}	3.0x10$^{-3\,ab}$	0.06^{a}	1.96^{a}	3.09^{a}

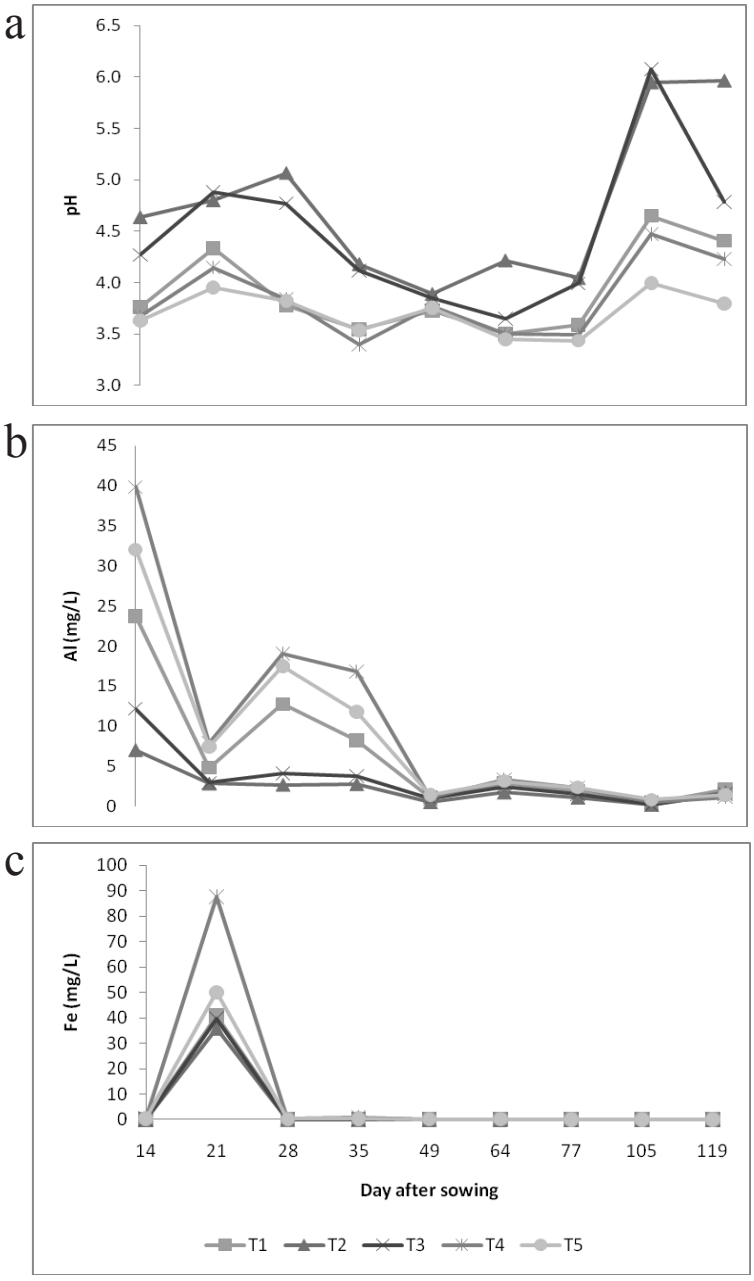


Fig.3: Changes in water pH (a) Al (b) and Fe (c) in the first season with time

up to expectation, below the national average of 3.8 t ha^{-1}. The highest concentration of Fe was found at 21 DAS (Fig.3c). Fig.5 shows the relationship between water pH and Al (a) and pH and Fe (b) for the first season, which are presented by equation Y= -5.88x + 32.36 (R^2=0.40) and Y= -0.08x + 0.44 (R^2=0.35), respectively. As Al and Fe in the water increased, the pH decreased. When Al and Fe increased above their pKa, the metal precipitated to form their inert hydroxides.

Second Season

The non-significant yield difference between treatments can be attributed to the adverse effect of rice blast during the flowering stage (Table 3). The area received high amount of rainfall during that time (October, 2010-December, 2010) period and farmers faced difficulties to drain out the excess water, as shown in Fig.6. This situation had resulted in high humidity which attracted diseases and as such the rice yield for the second season was erratic (Fig.7).

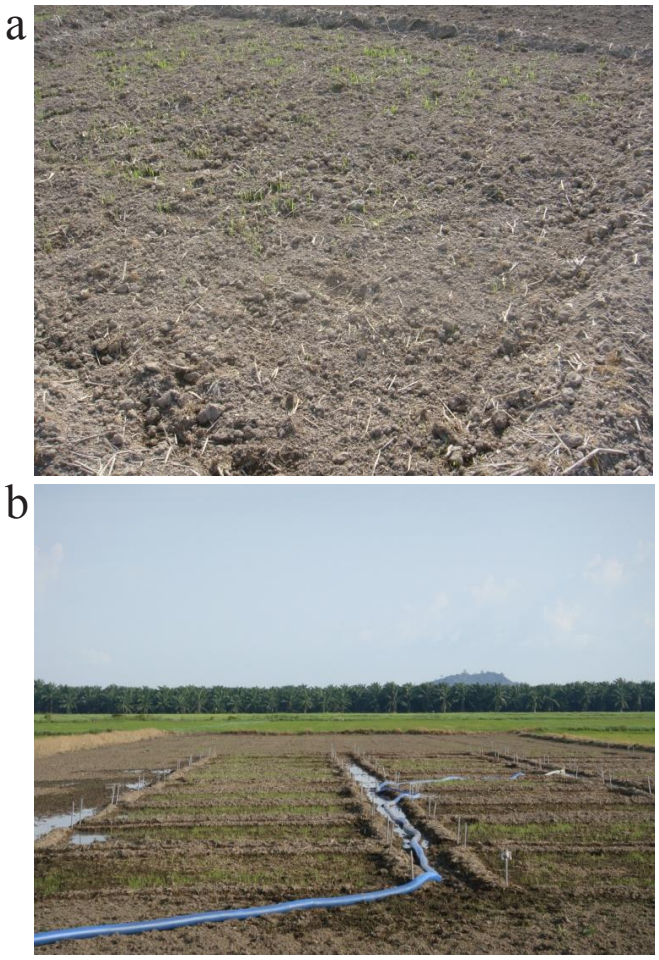


Fig.4: Dry condition during the first week after sowing (a) and water was pumped in from drainage canal (b) (for the first season)

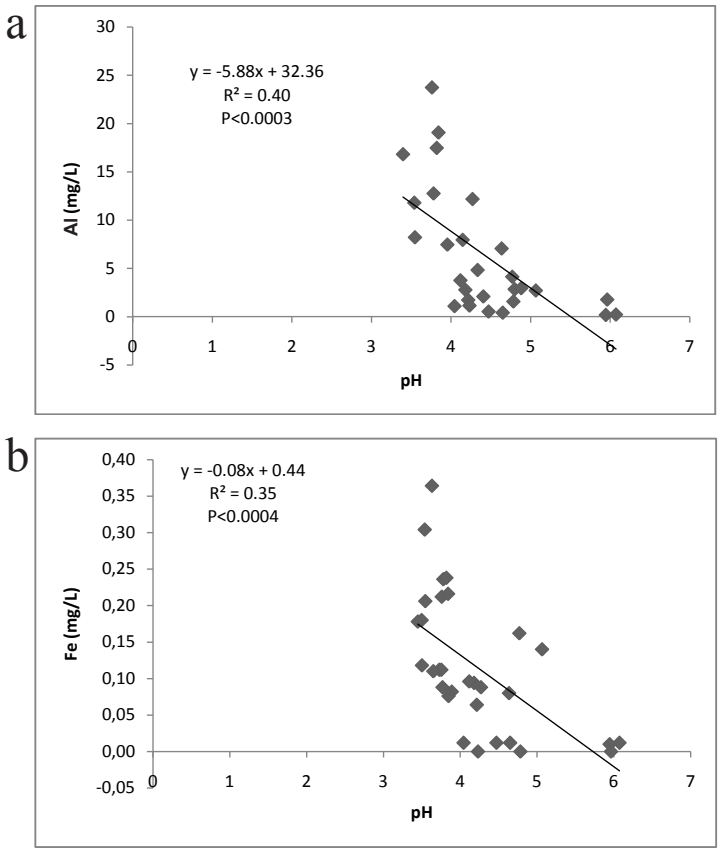


Fig.5: Relationship between water pH and Al concentration (a) and water pH and Fe concentration during the first season

Fig.6: The field condition during the second season with excess water

Rice blast is caused by an ascomycete fungus (*Magnaporthe grisea*). It spreads through spores and reproduces on its own. Thus, this disease spreads quickly in the infested paddy field. *M.grisea*, in some instance, has been named as *Magnaporthe oryzae*, *Pyricularia grisea* and *Pyricularia oryzae*. All these names are acceptable because scientists have yet to agree on a single name as it has different symptoms at different localities. Besides that, members of the *M. grisea* complex can also infect other cereal crops such as wheat, rye and pearl millet causing blast disease (Scardaci, 2003). Rice blast fungus causes economically significant crop losses annually in at least 85 countries worldwide. It is estimated to destroy enough rice to feed more than 60 million people (Scardaci, 2003; Crop Protection Compendium, 2011).

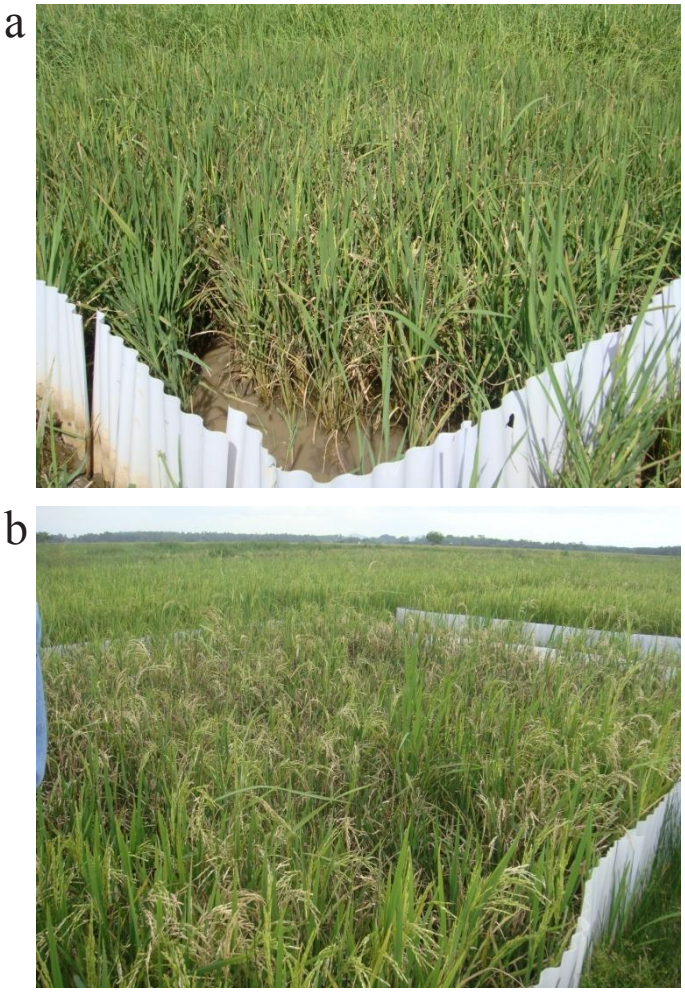


Fig.7: The condition of rice after being attacked by rice blast at 75 DAS (a) and 90 DAS (b) (the second season)

In the paddy field of the current trial, this disease started to attack the rice at 75 DAS. According to Yashida and Parao (1976), Ou (1985) and Scardaci (2003), rice blast is well known to cause severe yield losses in rice production systems. This scenario was noted to occur when cloud cover is high leading to low solar radiation. With low solar radiation, humidity often increases significantly and so does the rice blast infection. The infection rate of rice blast in paddy field tend to increase directly with increase in humidity as found by Dobermann and Fairhust (2000). Besides that, based on a study in multi-locations (Korea, Japan and China), Luo *et al*. (1998) found that changes of temperature between +3^{o}C in the ambient air show significant rice yield losses directly due to severe rice blast.

It is postulated that, the rice yield would have been higher than national average of 3.8 t/ha/season of rice yield if the paddy fields had not been attacked by the disease. In order to eliminate the disease, it is necessary to apply fungicide (a type of pesticide). However, usage of high amount of fungicide posses risk of environmental pollution and affects the farmers health as found in Vietnam (Hakan Berg, 2001), hence controlled usage of fungicide has to be practiced by the farmers. According to the farmers from the area, rice blast infested their paddy field every year with different degrees of severity. Therefore, a practical alternative is to use rice variety that is tolerant to the disease, which requires investment in terms of money and infrastructures.

Table 5 shows that the soil pH was still below 5 after the second harvest even after the application of 4 t GML ha^{-1}. Therefore, it was noted that the applied treatments did not alleviate the soil pH to the desired level of pH 6, and hence, not sufficient enough for good rice growth. Besides that, there was no significant difference for the concentrations of Al and Fe between the treatments (Table 5). It was noted that Al tended to decrease and Fe tended to increase over season. Al and Fe often precipitated as Al and/or Fe oxides and/or hydroxides in the soil. Al does not show prominent coloration in solution compared to Fe. In this trial, Fe was often observed as *'rust water'* within the nearby water-canal. This water is visually found to represent the iron reddish colour seeping from soil to the soil solution. Hence, oxidation-reduction processes that took place in a high acidity soils such as an acid sulphate soil also influence the Fe toxicity of the soil. This phenomenon has been described by Shamshuddin (2006) and Tan (2008).

Fig.8 shows the changes in water pH, Al and Fe concentration with time in the second season. The sampling was stopped at 77 DAS as the paddy field started to dry up. Application of GML and hydrated lime did not increase the pH and decrease the Al and Fe concentration as compared to the control. Likewise, liquid lime had no effect on pH. Fig.9 shows that Al concentration decreased with increasing pH and the equation is given by $Y= -0.25x + 1.97$ (R^2=0.53). This means that the pH needs to be increased in order to eliminate Al from damaging rice in the field.

Phosphorus Deficiency

Phosphorus (P) is mostly available for plant uptake when the soil pH is between 6.0 to 6.5, and decreases outside this pH range. In the study area (Merbok), the pH levels were less than 3.5, which are categorized as low soil pH (a.k.a high acidity). Besides that, these soils have high content of iron (Fe), as shown in Table 1.

When the soil is flooded, ferric (Fe) phosphate is converted to ferrous phosphate, which is more soluble in water, through a process called reduction process. The rate at which this process occurs, governs the amount of available P in the soil. On low pH soils, such as the Merbok soil, this reaction occurs quickly compared to alkaline soils. Thus, when the soil is flooded, the amount of P in solution increases available P for plant uptake. While P deficiency may seem to be present in Merbok soil soon after flooding; sufficient P may be released later in the season to produce better rice yields. When the soil is drained and the soil dries, P may again form compounds that are less soluble than prior to flooding.

It is stated by Dobermann and Fairhurst, (2000) that rice needs between 7 to 20 mg kg^{-1} of P for good rice growth. In this study, it was found that the available P at harvest was less than 3 mg kg^{-1} and there was no significant difference among the treatments. However, rice growth was not significantly affected by the low available P (Table 1), but reduction in the rice yield in the second season was prominent due to rice blast. Therefore, it was likely that P was immobilized by Al and Fe present in the

TABLE 5
pH, CEC, exchangeable bases (K, Ca, Mg, Al) and Fe of the soil

Sampling	Treatments	pH	CEC ($cmol_c\ kg^{-1}$)	Exchangeable bases ($cmol_c\ kg^{-1}$)				Fe (mg kg^{-1})
				K	Ca	Mg	Al	
1st (Before rice planting during first season on April 2010)	T1	3.14^{a}	11.73^{a}	0.13^{a}	0.15^{a}	2.69^{a}	10.96^{a}	222.48^{a}
	T2	3.18^{a}	13.96^{a}	0.37^{a}	0.58^{a}	3.16^{a}	10.56^{a}	215.15^{a}
	T3	3.22^{a}	14.10^{a}	0.18^{a}	0.63^{a}	3.24^{a}	11.27^{a}	214.91^{a}
	T4	3.10^{a}	12.20^{a}	0.15^{a}	0.39^{a}	2.87^{a}	11.01^{a}	196.95^{a}
	T5	3.05^{a}	15.07^{a}	0.16^{a}	0.35^{a}	3.12^{a}	12.03^{a}	176.31^{a}
2nd (After first harvest on August 2010)	T1	3.17^{bc}	15.57^{ab}	0.13^{b}	0.51^{abc}	2.81^{b}	7.27^{a}	333.32^{a}
	T2	3.25^{ab}	19.07^{a}	0.15^{ab}	0.70^{ab}	3.39^{a}	8.35^{a}	309.52^{a}
	T3	3.36^{a}	14.03^{b}	0.18^{a}	0.77^{a}	2.94^{b}	7.29^{a}	281.97^{a}
	T4	3.03^{c}	14.41^{b}	0.15^{b}	0.37^{bc}	2.96^{b}	8.68^{a}	264.45^{a}
	T5	3.00^{c}	15.29^{ab}	0.16^{ab}	0.33^{c}	3.07^{b}	8.73^{a}	198.52^{a}
3rd (After second harvest on February 2011)	T1	3.12^{b}	13.90^{ab}	0.11^{a}	0.60^{b}	3.01^{b}	6.74^{a}	358.36^{a}
	T2	3.33^{a}	15.31^{a}	0.13^{a}	0.98^{a}	3.99^{a}	6.43^{a}	371.96^{a}
	T3	3.13^{b}	13.30^{b}	0.13^{a}	0.95^{a}	3.27^{b}	6.14^{a}	365.93^{a}
	T4	3.07^{b}	13.66^{ab}	0.11^{a}	0.49^{b}	3.15^{b}	6.87^{a}	335.18^{a}
	T5	3.09^{b}	14.29^{ab}	0.11^{a}	0.45^{b}	3.07^{b}	6.84^{a}	316.50^{a}

Means followed by the same letter within a column are not significantly different (LSD's test, $P > 0.05$)

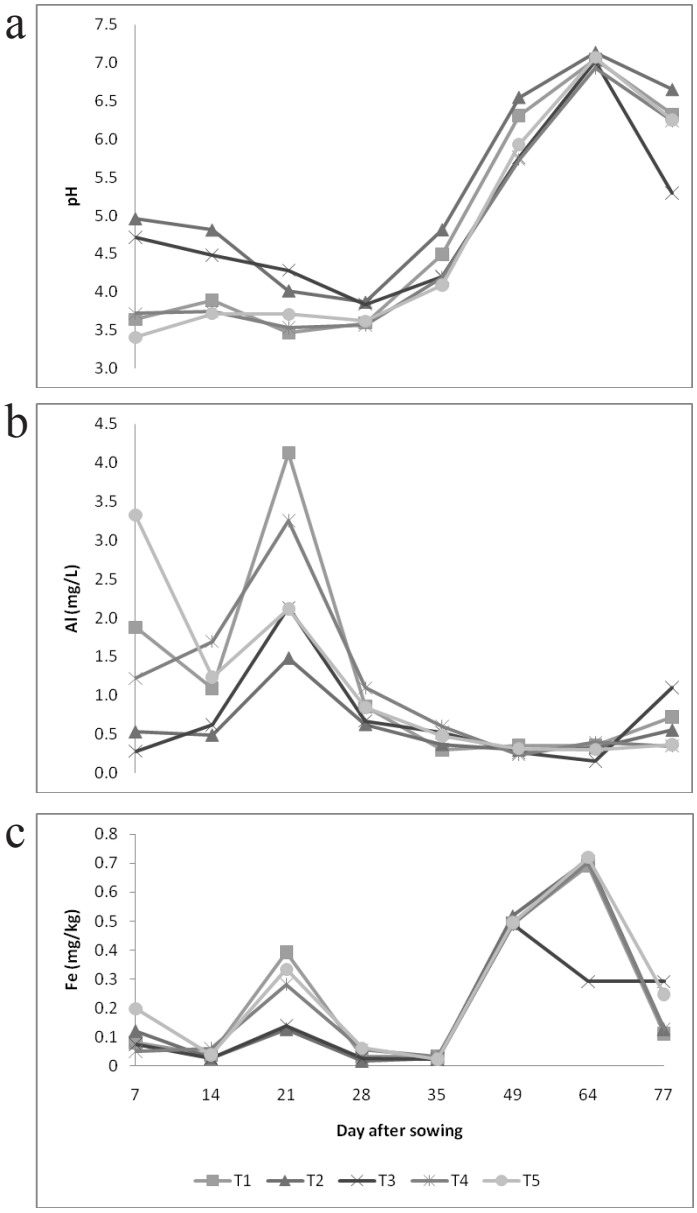


Fig.8: Changes in water pH (a), Al (b) and Fe (c) in the second season with time

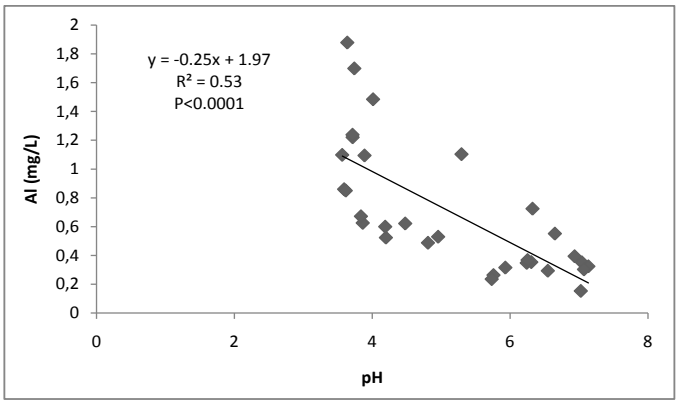


Fig.9: Relationship between water pH and Al concentration during the second season

soil via the formation of insoluble $AlPO_4$ or $FePO_4$ after the soil is drained and dries during harvest.

GENERAL DISCUSSION

Agronomic Practices

Several management and cultural practices can be used to improve the area and to increase rice production. The options include improving water management to irrigate and drain excess water, use of Al-tolerant rice variety and enhance soil fertility. In Merbok, the area used for rice cultivation is about 3000 ha. There is a potential to increase rice yield above the national average of 3.80 t ha^{-1} if an effective system of irrigation and drainage is put in place. Formerly, the area was occupied by high tidal mangrove flats and was converted to paddy field in 1964. The annual rainfall is 2155 mm with a pronounced dry period in December-March with evapo-transpiration rate exceeding rainfall (Ting *et al*., 1993). Another option is that Merbok River, which is approximately 2 km from the study area, can be utilized as a water source to irrigate the paddy field (Fig.5). Otherwise, the paddy field should get water from the nearby Muda Agricultural Development Authority (MADA) which has established irrigation and drainage system covering 96,000 ha to enable double cropping of rice.

Temperature and water source are the two major constraints in rice production, inclusive in Merbok (Kedah). Temperature at the study area varied highly from 32 to 50^{o}C and water source was scarce. High temperature may lead to heat stress mechanism in crop. This mechanism involves rolling in leaf to reduce moisture loss, thus reducing their yield (Ohta & Kimura, 2007). Meanwhile, scarcity of water adds to the lack of medium for nutrient mobilization and uptake. Therefore, one possible solution is to continuously pond the water during primary (March to June) and secondary (August to November) rainy season in Merbok (Kedah). This method is also suggested by Ikehashi (2007) as a good practice to improve water scarcity in rice field area. The pond water can be later

used to provide the best growth condition for rice root development during growth stage thus increasing possibility of high rice yield. After planting, flooding would also help suppress weed growth, improves the efficiency of nitrogen (Cassman *et al*., 1998) and in some environments, helps to protect the crop from high fluctuations in temperature. Meanwhile, Yang *et al*. (2004) found that continuous water logging prior to root development decrease root development and its activity. Fertilizer uptake by crop may also be affected. Plus, Kirk and Bouldin (1991) reported suppressive effects on rice root systems that reduce the yield under continuous flooding practice. Therefore, continuous flooding of rice paddy field is best avoided. These scenarios suggest that field capacity water is much preferred during vegetative stage for rice seed to imbibe the water and germinate well.

Many rice varieties are available in the market (i.e., MR 219, MR 220, MR 253 and etc.); however, MR 219 is often used by the farmers in Merbok (Kedah) and also used in this study. MR 219 has some tolerance to Al toxicity although it also shows low resistance to rice blast. Besides that, high amount of Al was detected in the water. This scenario creates another problem for rice production in the area. As such, another option is to breed Al-tolerant cultivars. Recently, Malaysia has released MR 256 variety, which is known to be acid-tolerant. Planting Al-tolerant cultivar would accumulate less Al in their foliage and subsequently the uptake of Ca and P is efficient even in the presence of high Al concentration in the water of the paddy field.

Planting time can be delayed after application of lime and flooding until the pH increases due to reduction of Fe (III) to Fe (II). The same reason is given for the satisfactory growth of oil palm seedlings grown on acid sulphate soils under flooded conditions (Auxtero & Shamshuddin, 1991). The application of 4 t GML ha^{-1} on an acid sulphate soils before rice planting only managed to raise the pH to about 4.5 (Shamshuddin, 2006). Liming at higher rate than this can become uneconomical for the farmers as shown in Table 6. The soluble Al and Fe will decline, while the exchangeable Ca and Mg will increase after liming. In addition, a study had reported that the toxic effect of Al can be reduced by the presence of Ca and Mg (Bohn *et al*., 1979). Likewise, Sanchez (1976) found that Al toxicity can be reduced somewhat by the presence of extra calcium and magnesium.

Adding GML would increase the soil pH with the addition of Ca and Mg into the soil. GML will ameliorate acid soil according to the following reactions:

$$(Ca, Mg)(CO_3)_2 \rightarrow Ca^{2+} + Mg^{2+} + 2CO_3^{2-} \quad \text{(equation 1)}$$

$$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^- \quad \text{(equation 2)}$$

$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \quad \text{(equation 3)}$$

GML dissolves readily on applying it into the acidic soil, releasing Ca and Mg (equation 1), and these macronutrients could be taken up by the growing rice

plants. Subsequently, the hydrolysis of CO_3^{2-} (equation 2) would produce hydroxyls that neutralize Al by forming inert Al-hydroxides (equation 3).

Farmers in Merbok are provided with subsidized fertilizers, pesticides and seeds by the Malaysian government for every planting season. Besides that, better link between farmers-government-extension officers-industry players are needed. Drum seeders in Bangladesh and India is a technology that saves labour and increases rice yield. This technology is known to be farmer-friendly, easy to use and practical to be applied in the field. Such improvement in rice cultivation in Malaysia can help the farmers to save time and reduce cost of production. The drum seeder consists of a series of perforated drums supported between two wheels and the seeds are placed in the drums and the device is hand-pulled by one farmer, allowing seeds to fall in rows into the puddled rice field (Kumar *et al*., 2009) compared to the scattered pattern of rice from broadcasting method. Through this practice, at least 10% increases in rice yield (Kumar & Ladha, 2011) were observed compared to the current production system.

Fertilizers should be applied according to the requirement of rice plants and it should be based on the recommended rate and have to be applied at the right time. This practice would help decrease pest infestation so that less pesticide is used and this helps reduce water pollution. Furthermore, it would help farmers reduce their production cost, while increasing the rice yield. Agronomists should help educate and guide the farmers in the management practices.

Cost Analysis

In order to increase the farmers' income and reduce production cost, a cost analysis is presented (Table 6). Table 6 shows that the application of 4 t GML ha^{-1} is the most expensive among the others, valued at USD 382 and resulted in the highest rice yield (3.50 t ha^{-1}) for the first season. Favourable water pH for rice growth is 6 and to raise the pH of acid sulphate soils to the desired level, it requires more than 4 t GML ha^{-1}, which is too costly. According to the record, rice yield in Merbok can be increased from <2 to 4.5 t ha^{-1} after annual liming at 2 t GML ha^{-1}

TABLE 6
Cost of different types of liming materials with labour

Rate	4 t ha^{-1} GML	2 t ha^{-1} hydrated lime	20 L ha^{-1} liquid lime (only 1st season)	20 L ha^{-1} liquid lime (1st and 2nd season)
Price	USD 50 t^{-1} = USD 200	USD 140 t^{-1} = USD 279	USD 97/20L = USD 97	USD 97/20L = USD 194
Labor	USD 46 =USD 182	USD 45 t^{-1} =USD 90	USD 16 ha^{-1} =USD 16	USD 16 ha^{-1} = USD 32
Total	USD 382 USD 3,820*	USD 369 USD 3,690*	USD 113 USD 1,130*	USD 226 USD 2,260*

*Average paddy land size is 10 ha^{-1} $farmer^{-1}$

(Ting *et al*., 1993). However, application of lime annually incurs labour cost and time consuming. Thus, a simple economics dictate here, as cost increase, profit margin decrease. Rice yield in Merbok (± 2 t ha^{-1} $season^{-1}$) is already lower than national level of 3.8 t ha^{-1}, hence farmers profit is quite low. With increase in production cost, most farmers may be reluctant to continue growing paddy. Currently, farmers in Merbok are using 2 t ha^{-1} of hydrated lime for every two season for rice production. And, with combination of direct drum-seeding method in Merbok, rice yield is expected likely to increase significantly.

CONCLUSION

Using ground magnesium limestone (GML) and hydrated lime at appropriate rate, rice cultivated on acid sulphate soils can yield comparable to that of the granary areas of Malaysia. This study showed that rice yield can be as high as 3.50 t ha^{-1} $season^{-1}$ even though it was subjected to drought and disease infestation. This yield was achieved by applying 4 t GML ha^{-1} although it cost USD 382 to the farmers. One ton of rice sold at the market price of USD 318. At this rate of lime application, the ameliorative effect can last for 2 seasons. In order to improve rice yield in Merbok, it is suggested that canal-water management and direct drum-seeding are applied through knowledge transfer from researchers to the rice farming community. Hence, it is believed that acid sulphate soils can be used productively for rice production so that self-sufficiency level (SSL) in Malaysia can be increased significantly, at least by 10-20% ha^{-1} $season^{-1}$.

ACKNOWLEDGEMENTS

We like to thank Universiti Putra Malaysia, Merbok Farmers' Organisation and farmers, for providing the experimental plots, the Ministry of Science, Technology and Innovation, Malaysia, for financial support through grant No. 05-01-04-SF-1094 and the Ministry of Higher Education Malaysia (under LRGS Programme – Food Security) for financial and technical support.

REFERENCES

Alva, A. K., Asher, C. J., & Edwards, D. G. (1986). The role of calcium in alleviating aluminum toxicity. *Aust. J. Soil. Res., 37*, 375-383.

Auxtero, E. A., & Shamshuddin, J. (1991). Growth of oil palm (*Elaeis guineensis*) seedlings on acid sulfate soils as affected by water regime and aluminum. *Plant Soil 137,* 243-257.

Bohn, H. L., McNeal, B. L., & O'Connor, G. A. (1979). *Soil chemistry*. John Wiley & Sons Inc. New York, p. 329.

Bouman, B. A. M., & Tuoang, T. P. (2001). Field water management to save water and increase its productivity in irrigated rice. *Agric. Water. Manag., 49*, 11-30.

Brady, N. C. (1974). *The nature and properties of soils* (8th Edition). New York.

Cassman, K. G., Peng. S., Olk, D. C., Ladha, J. K., Reichardt, W., Dobermann, A., & Singh, U. (1998). Oppurtunities for increased nitrogen-use efficiency from improved resource management in irrigated rice systems. *Field Crops Research 56,* 7-39.

Cate, R. B., & Sukhai, A. P. (1964). A study of aluminum in rice soils. *Soil Science, 98*, 85-93.

Carter, M. D. (1993). Soil sampling and methods of Analysis. In M. D. Carter (Ed.), *Canadian Society of Soil Science* (pp. 239-246). Florida USA: Lewis Publishers.

Chapman, H. D. (1965). Determination of cation exchange capacity. In C.A. Black (Ed), *Methods of soil analysis*, *2*(1), 891-900. Agronomy Monogr. 9. ASA, Madison, WI.

Chen Song., Sheng-guan, C., Chen Xin., & Guo-ping, Z. (2009). Genotypic differences in growth and physiological responses to transplanting and direct seeding cultivation in rice. *Rice Science, 16,* 143-150.

Crop Protection Compendium (2011). CAB International–online webpage access. Retrieved from http://www.cabi.org/cpc/

Dent, D. (1986). *Acid Sulfate Soils: A Base Line for Research and Development.* Wageningen, The Netherlands.

Dobermann, A., & Fairhust, T. (2000). *Rice: Nutrient Disorders and Nutrient Management*. Phosphate Institute of Canada and International Rice Research Institute, Las Banos, The Phillippines.

Elisa Azura, A., Shamshuddin, J., & Fauziah, C.I. (2011). Root elongation, root surface area and organic acid exudation by rice seedling under Al^{3+} and/or H^{+} stress. *American Journal of Agricultural and Biological Sciences, 6,* 324-331.

Enio, M. S. K., Shamshuddin, J., Fauziah, C.I., & Husni, M. H. A. (2011). Pyritization of the coastal sediments in the Kelantan plains in the Malay Peninsula during the holocene. *American Journal of Agricultural and Biological Sciences, 6,* 393-402.

Godbold, D. L., Fritz, E., & Huttermann, A. (1988). Aluminum toxicity and forest decline. *Proc. Nat. Acad. Sci., 85*, 3888-3892.

Hakan, B. (2001). Pesticide use in rice and rice-fish farms in the Mekong Delta, Vietnam. *Crop Protection, 20*, 897-905.

Horst, W. J., Rangel, A. F., Eticha, D., Ischitani, M., & Rao, I. M. (2009). Aluminum toxicity and resistance in *Phaseolus vulgaris* L. Physiology drives molecular biology. In H. Liao, X. Xian, & L. Kochian (Eds.). Proc. 7th Int. Symp. on Plant-Soil at Low pH (pp. 53-54). South China University of Technology Press.

Ikeda, H., Kano, H., & Kageyama, M. (1979). Effects of transplanting on the growth of muskmelons, and the redistribution of nitrogen absorbed in the nursery. *Scientia Horticulture 11*, 329-335.

Ikehashi, H. (2007). The Origin of Flooded Rice Cultivation. *Rice Science 14*, 161-171.

Kirk, G. J. D., & Bouldin, D. R. (1991). Speculations on the operation of the rice root in relation to nutrient uptake. In F.W.T. Penning de Vries (Ed.), *Simulation and system analysis for rice production* (pp. 195-203). Pudoc, Wageningen.

Kumar, V., & Ladha, J. K. (2011). Chapter Six-Direct Seeding of Rice: Recent Developments and Future Research Needs. *Advances in Agronomy, 111*, 297-413.

Luo, Y., Teng, P. S., Fabellar, N. G., & TeBeest, D. O. (1998). Risk analysis of yield losses caused by rice leaf blast associated with temperature changes above and below for five Asian countries. *Agric. Ecosyt. Environ., 68*, 197-205.

Mahajan, G., Bharaj, T. S., & Timsina, J. (2009). Yield and water productivity of rice as affected by time of transplanting in Punjab, India. *Agricultural Water Managament, 96*, 525-532.

Muhrizal, S., Shamshuddin, J., Husni, M. H. A., & Fauziah, I. (2003). Alleviation of aluminum toxicity in an acid sulfate soil in Malaysia using organic materials. *Commun. Soil Sci. Plant Anal., 34*, 2993-3012.

Muhrizal, S., Shamshuddin, J., Fauziah, I., & Husni, M.H.A. (2006). Changes in an iron-poor acid sulfate soil upon submergence. *Geoderma, 131*, 110-122.

Naklang, K., Fukai, S., & Nathabut, K. (1996). Growth of rice cultivars by direct seeding and transplanting under upland and lowland conditions. *Field Crops Research, 48,* 115-123.

Ohta, S., & Kimura, Ai. (2007). Impacts of climate changes on the temperature of paddy waters and suitable land for rice cultivation in Japan. *Agricultural and Forest Meteorology, 147*, 186-198.

Ou, S. H. (1985). *Rice diseases* (2nd Edn.). Commonwealth Mycologi-cal Institute, Kew, Surrey, England, p. 380.

Paramananthan, S. (1987). *Field legend for soil surveys in Malaysia*. Serdang, Malaysia: UPM Press.

Park, Y. D., & Kim, Y. S. (1970). The effect of wollatonite and manganese dioxide on rice grown on a flooded acid sulphate soil. *Journal of Korean Society of Soil Science and fertilizer, 3*(1), 23-28.

Ponnamperuma, F. N., Attanandana, T., & Beye, G. (1973). Amelioration of three acid sulfate soil for lowland rice. *Proc. Int'l Symp. Acid Sulfate Soils, ILRI PUBL. 18*, Wageningen II, pp. 391-406.

Prasanna Kumar, G. V., Srivastava, B., & Nagesh, D. S. (2009). Modeling and optimization of parameters of flow rate of paddy rice grains through the horizontal rotating cylindrical drum of drum seeder. *Computer and Electronic in Agriculture, 65*, 26-35.

Ridolfi, M., & Garrec, J. P. (2000). Consequences of the excess al and a deficiency in ca and mg for stomatal functioning and net carbon assimilation of beech leaves. *Ann. Sci., 57*, 209-218.

Sanchez, P. A. (1976). *Properties and management of soil in the tropics.* New York: John Wiley & Sons Inc. p. 618.

Scardaci, S. C. (2003). *A New Diseases in California.* University of California-Davis: Agronomy Fact Sheet Series 1997-2.

Shamshuddin, J., & Auxtero, E. A. (1991). Soil solution composition and mineralogy of some active acid sulfate soil as affected by laboratory incubation with lime. *Soil Sci., 152,* 365-376.

Shamshuddin, J., Jamilah, I., & Ogunwale, J. A. (1995). Formation of hydroxyl-sulfates from pyrite in coastal acid sulfate soil environments in Malaysia. *Commun. Soil Sci. Plant Anal., 26,* 2769-2782.

Shamshuddin, J., Muhrizal, S., Fauziah, I., & Van Ranst, E. (2004a). A laboratory study of pyrite oxidation in an acid sulfate soils. *Commu. Soil. Sci. Plan. Anal., 35,* 117-129.

Shamshuddin, J., Muhrizal, S., Fauziah, I., & Husni, M. H. A. (2004b). Effects of adding organic materials to an acid sulfate soil on growth of cocoa (*Theobroma cacao* L). *Sci Total Environ., 323*, 33-45.

Shamshuddin, J. (2006). *Acid Sulfate Soils in Malaysia*. Serdang: UPM Press.

Shazana, M. A. R. S., Shamshuddin. J., Fauziah, C. I., & Syed Omar, S. R. (2011). *Alleviating the infertility of an acid sulphate soil by using ground basalt with or without lime and organic fertilizer under submerged condition*. Land Degrad Dev. In press.

Soil Survey Staff. (2010). *Keys to Soil Taxonomy*. United States Department of Agriculture, Washington, DC.

Susan, C. M., Hue, N. V., & Michael, A. D. (2007). *Handbook of Plant Nutrition*. London.

Suswanto, T., Shamshuddin, J., Syed Omar, S. R., Peli Mat., & Teh, C. B. S. (2007). Alleviating an acid sulfate soil cultivated to rice (*Oryza sativa*) using ground magnesium limestone and organic fertilizer. *Soil Environ., 9*, 1-9.

Tan, K. M. (2008). Environmental Soil Science. In K. H. Tan (Ed.). CRC Press, Georgia, USA.

Tan, K., & Keltjens, W.G. (1995). Analysis of acid soil stress in sorghum genotypes with emphasis

on aluminum and magnesium interactions. *Plant and Soil, 171*, 147-150.

Ting, C. C., Rohani, S., Diemont, W. S., & Aminuddin, B. (1993). The development of an acid sulfate area in former mangroves in Merbok, Kedah, Malaysia. In D. L. Dent, M. E. F. & Van Mensvoort (Eds.), *Selected Papers of the Ho Chi Minh City Symposium on Acid Sulfate Soils* (pp. 95-101). Wageningen, the Netherlands.

Van Breemen, N., & Pons, L. J. (1978). Acid sulfate soil and rice. In IRRI (Eds.), *Soils and Rice* (pp. 739-761). International Rice Research Institute, Philippines.

Yang, C., Yang, L., Yang, Y., & Ouyang, Z. (2004). Rice root growth and nutrient uptake as influenced by organic manure in continuously and alternately flooded paddy soils. *Agric. Water. Manag., 70*, 67-81.

Yashida. S., & Parao, F. (1976). *Climate influence on yield and yield components of lowland rice in the tropics. Climate and Rice.* International Rice Research Institute, Philippines, pp. 471 – 494.

Zhu, Y., Di, T., Chen, X., Yan, F., & Schubert, S. (2009). Adaptation of plasma membrane H^+-ATPage of rice roots to low pH. Proc. 7th Int. Symp. on Plant-Soil at Low pH (pp. 124-125. In H. Liao, X. Xian, & L. Kochian (Eds.), South China University of Technology Press.

Pertanika J. Trop. Agric. Sci. 37 (2): 249 - 261 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Cattle Grazing Effect on *Mimosa pudica* L. in Tropical Pasture System

Majid Ajorlo[1]*, Ramdzani Abdullah[2], Ridzwan Abdul Halim[3] and Mahboubeh Ebrahimian[4]

[1]*Faculty of Natural Resources, University of Zabol, 98615 Zabol, Iran*
[2]*Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[4]*Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

ABSTRACT

Mimosa pudica is the most abundant and problematic invasive species in tropical pastures. This study investigates the effects of two cattle grazing systems, long-term moderate grazing (LTG, 2.7 animal unit/ha/yr for 33 years) and short-term heavy grazing (STG, 5 animal unit/ha/yr for 2 years) compared with ungrazed exclosure pasture on the invasion of *M. pudica* and its relationship with herbage production in tropical pasture. *M. pudica* and pasture production were concurrently sampled four times at the end of grass growing period at both grazed and ungrazed pastures. Mean density of *M. pudica* was 56% greater ($P<0.05$) in the LTG pasture than that in the ungrazed exclosure although it did not vary ($P>0.05$) between the STG pasture and ungrazed exclosure. Mean importance value (IV) of *M. pudica* in the LTG pasture was 46% lower ($P<0.05$) than that in the ungrazed exclosure, and this was 220% greater ($P<0.05$) in the STG pasture than that in the ungrazed exclosure. Pasture herbage production was unrelated ($P>0.05$) to the density, IV and dry matter (DM) of *M. pudica* in either pasture system. An insignificant negative relationship was found between the density and DM of *M. pudica* with pasture production. In contrast, a positive but insignificant relationship was observed between %IV of *M. pudica* and pasture production in both pasture sites. The LTG system had adverse effect on *M. pudica* population, whereas STG system supplied relatively desirable conditions for *M. pudica* establishment and infestation.

Keywords: heavy grazing, invasive species, Malaysia, *Mimosa pudica*, moderate grazing, pasture production

ARTICLE INFO

Article history:
Received: 21 March 2013
Accepted: 4 December 2013

E-mail addresses:
ajorlo_m54@yahoo.com (Majid Ajorlo),
ramdzani@upm.edu.my (Ramdzani Abdullah),
ridzwan@upm.edu.my (Ridzwan Abdul Halim),
m_ebrahimian81@yahoo.com (Mahboubeh Ebrahimian)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Sensitive plant (*Mimosa pudica* L.) is a small, perennial and ligneous invasive species which is widely distributed in tropical regions, especially in south-eastern Asia (Galinato *et al*., 1999) and has become a pantropical weed (Magda *et at*., 2006; Stur & Shelton, 1990). *M. pudica* blooms all year round and reproduces only by seed throughout the year in tropical regions. An adult plant can produce about 675 seeds/ plant/ year (Holm *et al*., 1997; Chauhan & Johnson, 2009a). The plant grows both vertically and horizontally, and an individual adult can cover an area of 1 m and 2 m, respectively. *M. pudica* is the most prevalent, abundant and problematic invasive species in either improved or native tropical pastures (Chauhan & Johnson, 2009a). This particular species can decrease both the quantity and quality of forage (Wardle *et al*., 1995), pasture lifetime and increase weeding and pasture renovation costs. Adult plants may decrease animal performance indirectly through impaired grazing of adjacent forage plants making grazing difficult because of its thorny structure (Grekul & Bork, 2004; Chauhan & Johnson, 2009a). This invasive plant is not grazed by cattle in its adult stage and may always be considered detrimental plant in pastures.

The problem of invasive woody species, *M. pudica*, in pastures is often unresolved (Magda *et al*., 2006). Prescribed burning, mechanical shredding, manual weeding and herbicides application are common strategies to control invasive species in pastures. However, these methods are rather complex to manage, expensive and have further adverse impacts on environmental resources. Meanwhile, manual weeding of spiny weeds is difficult as thorns penetrate and lacerate the hands. Burning can increase soil temperature which favours *M. pudica* seeds to release from dormancy and stimulate germination (Chauhan & Johnson, 2009b). Burning may encourage *M. pudica* to spread in pastures (Siregar *et al*., 1990). Chemical and mechanical control measures are financially and environmentally unsustainable in long term.

Simonet (1990) reported that grazing can reduce the dominance of *M. pudica* in pastures. Appropriate grazing intensity may slow down or control *M. pudica* population directly through ingestion of young seedlings biomass and reduction in the number of reproductive buds (Magda *et al*., 2006) and indirectly by hoof actions such as treading, sitting, pawing, jumping, and running only under appropriate grazing system (Harker *et al*., 2000). Despite the importance of *M. pudica* in tropical pastures, no published documents are available on the effects of cattle grazing on *M. pudica* population at present. The primary objective of this study was to determine the effects of cattle grazing on the attributes of *M. pudica* in tropical native and improved pastures with short-term heavy (for 2-year) and long-term moderate (for 33-year) rotational grazing systems, respectively. The secondary objective was to determine the relationships between the attributes of

M. pudica with pasture herbage production to predict pasture herbage loss. This was achieved by measuring density, importance value (IV) and dry matter (DM) production of *M. pudica,* as well as pasture herbage production. This study aimed to test the hypothesis that cattle grazing would affect the invasion of *M. pudica* and pasture herbage production would be related to *M. pudica* invasion.

MATERIALS AND METHODS

Study Area

This study was conducted at the Universiti Putra Malaysia Livestock Section, about 20 km south of Kuala Lumpur, Malaysia. Two study sites, Taman Pertanian Universiti (TPU) catchment (2º 58' 53" N; 101º 43' 38" E) and the Ladang site (3° 00' 28" N; 101° 42' 10" E), were assessed indicating a perennial improved and native pastures, respectively. The area has humid tropical climate; with mean annual rainfall of 2471 mm and mean annual temperature of 24.5°C.

The soil type was classified as *Typic Hapludox* (Munchong series) representing the Oxisols with >35% clay at the TPU catchment. The Ladang site is on a tin mine that had been abandoned in the 1950s and the soils are sandy clay in texture.

Vegetation of the TPU site consisted of improved pasture with a dominant cover of introduced tropical grasses such as signal grass (*Brachiaria decumbens* Stapf.) and guinea grass (*Panicum maximum* Jacq.) at the TPU site. The Ladang site, both grazed and ungrazed sites, was useless native grassland before establishment mainly dominated by carpet grass (*Axonopus compressus* (Sw.) Beauv.), hillo grass (*Paspalum conjugatum* Berg.) and slender panicgrass (*Ottochloa nodosa* Kunth). *M. pudica* was the only invasive species in both sites. The density of *M. pudica* in grazed and ungrazed pastures was 3.6 and 1.94 (plants/m^2) in the TPU site, whereas its density was 1.7 and 2.8 (plants/m^2) in grazed and ungrazed pastures of the Ladang site, respectively, before the initiation of grazing trials in this study.

The TPU site (180 ha) consists of improved pasture with introduced tropical grasses representing a long-term moderate (LTG, 2.7 animal unit (AU)/ha/year for 33 years) rotational grazing system with cattle from its establishment in 1975 and the Ladang site (4 ha) representing a short-term heavy (STG, 5 AU/ha/year for 2 years) rotational grazing systems with cattle since its establishment in 2007. The TPU site was divided into 9 paddocks ranging in sizes from 5 to 30 ha and the site was divided into four paddocks of similar size (1 ha). Grazing exclosures were also constructed in 1975 (20 ha) and 2007 (4 ha) at the TPU and Ladang sites, respectively. Exclosures are laid just beside the grazed paddocks on a terrain with similar soils and vegetation as the grazed area in both sites. In this study, breed, age and sex of cattle were Kedah-Kelantan (KK), about 5 years old and female, respectively. AU is defined as one mature local cow weighing about 250 kg with or without suckling calf. The rotational grazing system consisted of moving the cattle according to the forage

quantity into a new paddock for grazing. In this study, moderate grazing means 45-55% use of pasture herbage that allows the palatable species to maintain themselves. Heavy grazing involves 55-70% use of pasture herbage.

Experimental Design and Grazing Treatment

Each site was treated as an experiment. At the TPU site, four areas (6, 6.6, 8 and 9.5 ha) that had a homogeneous distribution of vegetation and uniform topography were selected in different paddocks. Each paddock at the Ladang site was considered to be a replicate. Four replicates were also defined in each grazing exclosure. The exclosures had no anthropogenic manipulation such as fertilizer application, ploughing and grazing since their construction and provided a control to compare the grazing effects. Therefore, for the TPU site, the treatments were (1) no grazing by cattle and (2) grazing at a moderate stocking rate under rotational grazing system for 33 years. For the Ladang site, the treatments included (1) no grazing by cattle and (2) grazing at a heavy stocking rate under rotational grazing system for 2 years.

Measurement of M. pudica Attributes

A combination of both systematic and randomized methods was used to measure the attributes of weed and forage plants. The systematic sampling design was used in the location of transect lines, while the random sampling design was used in establishing the quadrats (Kamaruzaman & Nik, 1992; Mesdaghi, 2004). A set of four 10 m transect lines spaced 100 m apart was established at each replicate at the TPU site, and two 10 m transect lines spaced 20 m apart were made at each replicate in the Ladang site. A quadrat (0.25 m^2) was randomly placed in each transect line. An exclosure cage technique was used to protect weed and pasture plants in grazed treatments (Mannetje, 1978). Both grazed and exclosures were sampled four times at the end of grass growing period in 2010. As such, measurement was performed every six weeks in the native pastures of the Ladang (namely, 19 March, 09 May, 06 July, 19 August 2010) and at eight-week intervals at improved pastures of the TPU site (namely, 13 May, 15 July, 04 September, 15 November 2010). Measurements in grazed treatment and in the exclosure were carried out in an identical way. No mechanical or chemical weeding operations were practiced to control invasive species population since establishment date in the Ladang site and one year before trial in the TPU site.

Mimosa pudica density, percent cover, importance value (IV), dry matter (DM) production and its proportion to pasture herbage production were determined across the four sampling events. After each sampling event, all transects were relocated systematically in new places within the replicate and quadrats were re-randomized on each transect. *M. pudica* density (plant/m^2) was determined by counting the number of individual plants separately within quadrats using equation 1 (Tauseef *et al.*, 2012). The percent cover

of *M. pudica* was assessed visually in each quadrat. Importance value of *M. pudica* presence in the pasture was also determined by calculating importance value percent (%IV) (Tracy & Sanderson, 2004). Percent IV gives an index of the relative importance of each species in the pasture by measuring its frequency and cover. Percent IV was calculated from the summation of relative frequency and cover values for *M. pudica* measured in the 0.25 m^2 quadrat using the equations 2 to 6 (Tracy & Sanderson, 2004; Pragada *et al*., 2011). Clipped *M. pudica* biomass in each quadrat was bagged in a perforated paper sack separately and subsequently was oven dried at 65°C for 48 hr to constant weight and weighed to determine DM production.

$$\text{Density (plants m}^2\text{)} = \frac{\text{Total number of individuals of a species in all quadrants}}{\text{Total numbers of quadrants}} \quad (1)$$

$$\text{Frequency (\%)} = \frac{\text{Numbers of quadrats in which species occured}}{\text{Total number of quadrats}} \times 100 \quad (2)$$

$$\text{Relative frequency} = \frac{\text{Frequency of individuals of a species}}{\text{total frequency of all species}} \times 100 \quad (3)$$

$$\text{Relative cover} = \frac{\text{cover of individual of a species}}{\text{total cover of all species}} \times 100 \quad (4)$$

$$\text{Importance value (\%)} = \frac{\text{Relative Frequency + relative cover}}{2} \quad (5)$$

Measurement of Pasture Herbage Production

In each quadrat, forage plants were cut to a 5 cm stubble height and subsequently hand-separated into live and dead material fractions. Green herbage was placed in a perforated paper bag and dried in a forced air oven at 65°C for 48 hr to constant weight and then weighed for DM determination (MAFF, 1986).

Statistical Analysis

For the analysis, it assumed that the replicates were independent. The treatments of the study were not replicated in space and it was made the assumption that replicates, which were nested within the treatments, provided an approximation of the experimental error. The present experimental approach was also used by other researchers (Frank *et al*., 1995; Wienhold *et al*., 2001; Liebig *et al*., 2006; Li *et al*., 2009). The use of pseudo-replications was justified based on the duration of given treatments that were greater than 2 years old. Site differences were expected due to differences in their management, pasture type, soil type and treatment period. Thus, TPU and Ladang sites were evaluated separately.

Assumptions of normality and homogeneity of variance were checked and log-transformed as appropriate. For log-transformed variables, the mean of the untransformed data was used to express central tendency and the standard error derived from log-transformed data was used to express precision. Multivariate analysis of variance (MANOVA) (SPSS release 16.0.1, SPSS Inc. 2007) was applied to assess grazing treatments effects on *M. pudica* attributes. Adjustment for multiple comparisons between means was done by Least Significant Difference (LSD) test. Differences were assessed at the significance level of $P<0.05$.

Additionally, the study was aimed to determine whether the density, %IV and

DM (independent variables) of *M. pudica* vary in their ability to present loss in pasture herbage production (dependent variable) and determining which variable has the most significant correlation with pasture herbage loss as explained by Grekul and Bork (2004). Relationships between the attributes of *M. pudica* and pasture production were analyzed by site using linear regression and Pearson correlation coefficient.

RESULTS

Grazing Effect on M. pudica Attributes

Cattle grazing had no effect ($P>0.05$) on the percent cover of *M. pudica* in either LTG or STG system (Tables 1 and 2). Nonetheless, the percent cover of *M. pudica* in LTG and STG grazing systems was about 96 and 36% lower ($P>0.05$) than that in the grazing exclosures, respectively, albeit insignificant.

The LTG system led to greater ($P<0.05$) density of *M. pudica* in the improved pasture (Table 1) but it did not vary between STG system and its ungrazed exclosure (Table 2). Meanwhile, the density of *M. pudica* in LTG system was 56% greater than that in ungrazed exclosure.

Percent IV of *M. pudica* in the LTG system was 46% lower ($P<0.05$) than that in the ungrazed exclosure (Table 1). In the STG system, however, it was 220% greater ($P<0.05$) than that in the ungrazed exclosure (Table 2).

TABLE 1
Effects of long-term moderate grazing system (LTG) on *Mimosa pudica* attributes in tropical improved pasture (TPU site)

M. pudica attribute	Grazed	Ungrazed	SE†	*F*	*P*
Cover (%)	3.16	7.50	0.292	0.31	0.57
Density (plants/m^2)	4.33a	2.78b	0.786	4.20	0.04
Importance value (%)	18.92a	34.98b	5.22	8.09	0.009
Dry matter (g DM/m^2)	12.82	24.50	0.234	0.012	0.98
Proportion to total herbage production (%)	12.67	17.33	0.222	0.057	0.80

Means in a row with unlike lower case letters significantly differ at $P<0.05$
†Standard error

TABLE 2
Effects of short-term heavy grazing system (STG) on *Mimosa pudica* attributes in tropical native pastures (Ladang site)

M. pudica attribute	Grazed	Ungrazed	SE†	*F*	*P*
Cover (%)	1.26	1.97	0.547	1.20	0.28
Density (plants/m^2)	4.00	3.20	1.23	2.03	0.16
Importance value (%)	45.67a	14.25b	5.40	28.72	00
Dry matter (g DM/m^2)	7.60	7.50	2.70	0.51	0.48
Proportion to total herbage production (%)	5.61	7.85	1.89	0.65	0.42

Means in a row with unlike lower case letters significantly differ at $P<0.05$
†Standard error

Dry matter production (g DM/m^2) of *M. pudica* was generally similar (P>0.05) between the grazed and ungrazed sites in either grazing system (Tables 1 and 2). The proportion (%) of *M. pudica* DM to pasture production did not vary (P>0.05) between the grazed and ungrazed sites in either grazing system (Tables 1 and 2). The results indicated that the proportion of *M. pudica* DM to pasture production in the grazed sites was lower than that in the ungrazed exclosures, albeit insignificant. For example, in the LTG system, *M. pudica* contribution to herbage production was about 27% lower than that in the ungrazed exclosure (Table 1).

Relationships between M. pudica Attribute and Pasture Production

Pasture production was unrelated (P>0.0.05) to *M. pudica* attributes such as density, percent IV and DM production in both the LTG and STG systems (Tables 3 and 4). An insignificant negative relationship was found between the density and DM of *M. pudica* with pasture production. In contrast, a positive but insignificant relationship was observed between %IV of *M. pudica* and pasture production in both pasture sites (Tables 3 and 4).

DISCUSSION

Grazing Effect on M. pudica Attributes

Relatively lower density of *M. pudica* in ungrazed exclosures compared with grazed pastures in both sites may be attributable to high accumulated litter and light competition. Accumulated litter can reduce seed germination and seedling emergence of plants through providing mechanical barrier, releasing allelochemicals and reducing light transmittance (Li *et al*., 2009; Chauhan & Johnson, 2009b). The mean value of litter biomass was 10.99 and 22.65 g/m^2 in the grazed pasture and ungrazed exclosure of the TPU site, and this was 10.79 and 18.2 g/m^2 in the grazed pasture and ungrazed exclosure of the Ladang site. On the other hand, ungrazed exclosures had tall pasture height and therefore competition for light

TABLE 3
Relationships between the attributes of *Mimosa pudica* (X) and pasture herbage production (Y) in tropical improved pastures (TPU site)

M. pudica attributes	Empirical relationship	*r*	*F*	*P*
IV	Y = 87.97 (±4.98) + 1.87 (±0.29) X	0.31	2.32	0.09 *
Density	Y = 157.81 (±24.25) – 3.81 (±9.55) X	–0.08	0.16	0.69 NS
DM	Y = 136.02 (±21.09) + 0.45 (±0.63) X	–0.15	0.51	0.48 NS

IV: importance value; DM: dry matter
Means (± standard error) are presented for equation intercepts (a) and slope (b).
r: Correlation coefficient. Correlations were either not significant (NS) or significantly different at P<0.10 (*), P<0.05 (**).

is greater in such pasture. Average pasture plants height was 35.4 and 74.8 cm in the grazed pasture and ungrazed exclosure of the TPU site, and 14.1 and 57.5 cm in the grazed pasture and ungrazed exclosure of the Ladang site. *M. pudica* is a short and shade intolerant plant. The amount of radiation received by juveniles of *M. pudica* in a tall grass pasture (at a height of about 50 cm), typically is low in lower layer (Magda *et al*., 2006). Magda *et al*. (2006) indicated that shading had significant negative effects on branching, mortality and flowering rate of seedlings of *M. pudica*. This species will be out-competed in such environment due to its inability to compete with tall grasses. Consequently, high competition for light in ungrazed exclosures might be another reason for low population of *M. pudica* in grazing exclosures. In Canada, Harker *et al*. (2000) found that increasing levels of cattle grazing intensity led to greater densities of shepherd purse (*Capsella bursa-pastoris*) and dandelion (*Taraxacum officinale*) in the perennial pastures.

The importance value (IV) was calculated to compare the ecological significance of *M. pudica* in pastures with and without cattle grazing (Tauseef *et al*., 2012). It provides knowledge on overall importance of each species in a plant community (Giliba *et al*., 2011). Greater IV of *M. pudica* in the STG system (45.67%) compared with ungrazed exclosure (14.25%) indicates that it has become more important species after 2 years of heavy grazing in native pastures of Ladang site. This finding highlights the ability of *M. pudica* to withstand defoliation and treading activities by cattle. *M. pudica* with high importance value in pastures of Ladang site has poor grazing value and it is an indicator of disturbance. However, lower IV of *M. pudica* in the LTG system (18.92%) compared with ungrazed exclosure (34.98%) indicates that moderate grazing was a more appropriate system in inhibiting *M. pudica* infestation in improved pastures of the TPU site. Consequently, different trend of *M. pudica* population, i.e. an upward trend in the STG system and a downward trend in

TABLE 4
Relationships between the attributes of *Mimosa pudica* (X) and pasture herbage production (Y) in tropical native pastures (Ladang site)

M. pudica attributes	Empirical relationship†	*r*	*F*	*P*
IV	Y = 96.45 (±7.32) + 0.82 (±0.63) X	0.27	1.66	0.10 NS
Density	Y = 122.94 (±18.02) + 1.95 (±4.52) X	−0.09	0.19	0.67 NS
DM	Y = 112.31 (±18.19) + 2.26 (±2.04) X	−0.23	1.23	0.27 NS

IV: importance value; DM: dry matter
Means (± standard error) are presented for equation intercepts (a) and slope (b).
r: Correlation coefficient. Correlations were either not significant (NS) or significantly different at $P<0.10$ (*), $P<0.05$ (**).

the LTG system can be related to direct and indirect impacts of cattle grazing.

Dry matter (DM) production of *M. pudica* in the LTG system was about two times lower than that in ungrazed exclosure (Table 1), indicating that the DM production of *M. pudica* was adversely affected by the LTG system. It is expected that the DM production of *M. pudica* should be reduced in native pasture of Ladang site due to heavy grazing by cattle for 2 years. However, Table 2 shows no difference between the grazed site and ungrazed exclosure with regard to DM production values. This indicates that even under heavy grazing condition *M. pudica* was able to produce dry matter as much as ungrazed exclosure. Consequently, it can be concluded that the STG system supplied relatively desirable conditions for DM production in *M. pudica*. Stur and Shelton (1990) also stated that *M. pudica* withstands heavy grazing pressure. Heavy grazing substantially weakens the perennial grasses (Harker *et al*., 2000). As cattle grazing pressure increases, grazing sensitive species become less abundant and are replaced by grazing tolerant and invasive species (Yates *et al*., 2000) which are more resistant to cattle trampling and better adapted to compacted topsoil (Martinez & Zinck, 2004).

Defoliation has a slight effect on seedling, juvenile mortality, and flowering rate of *M. pudica*, in comparison with shading (Magda *et al*. 2006). Defoliation can enhance branching and production of new branches in *M. pudica*, except when defoliation takes place after shading and high competition for light. The establishment and survival of seedlings of *M. pudica* is chiefly controlled by the indirect effect of grazing, i.e., hoof actions such as treading, sitting, pawing, jumping, and running (Harker *et al*., 2000). Appropriate grazing management system can influence *M. pudica* by creating competitive conditions by maintaining enough pasture plants height and/or facilitating population recruitment through removing adjacent grasses rather than direct defoliation by animals (Van Der Wal *et al*., 2000). In fact, competition is a factor that controls plant invasion particularly in nutrient limiting environments (Lopez-Zamora *et al*., 2004).

In heavily grazed pastures, the negative shading effect on juvenile plants of *M. pudica* decrease largely, as grazing opens the dense cover of pasture through removing upper parts of grass plants. Grazing intensity and duration are main determinants in residual pasture height and the extent of subsequent effect on juvenile plants. If pasture plants are grazed to ground surface, which happens in heavy grazing system, juvenile plants will not be subjected to hard competition for light from adjacent grass plants. This enhances favourable conditions for growth of young stages of this species and increase recruitment to *M. pudica* population. This issue was remarkable in native pastures of the Ladang site with heavy grazing system.

Additionally, *M. pudica* is a seismonastic plant in which the leaves (pinna) close and the petiole falls down in response to wind, vibration and touch as a defence

mechanism for protection from animals and insects (Volkov *et al*., 2010). Thigmonastic movement (response of a plant to touch) in *M. pudica*, associated with fast response to environmental stimuli such as animal biting and grazing, appear to enhance plant survival and establishment in pastures with heavy grazing.

Relationships between the Attributes of M. pudica and Pasture Production

In general, the relationships between *M. pudica* attributes and pasture production were insignificant in this study (Tables 3 and 4). Percent IV and density of *M. pudica* had the highest positive and the lowest negative insignificant relationships with pasture production in both pasture types, respectively (see Tables 3 and 4). Insignificant relationships between measured variables can be mainly related to small population and low magnitude of *M. pudica* in the studied pastures. However, this study revealed the negative relationship between *M. pudica* DM production and density with pasture production (Tables 3 and 4), indicating negative effects of biomass and numbers per unit area of *M. pudica* on tropical pasture production. In USA, Tracy and Sanderson (2004) and Ferrell *et al*. (2006) reported a negative relationship between weed density and herbage production. Ferrell *et al*. (2006) reported that bahiagrass (*Paspalum notatum*) herbage production reduced by increasing giant smutgrass (*Sporobolus indicus*) density. Hume (1985) stated that weed dry matter estimates relative spring wheat (*Criticum aestivum*) production loss better than density. Grekul and Bork (2004) results from perennial pastures showed no difference between Canada thistle (*Circium arevens*) density and DM production for predicting herbage production loss in pastures. Harker *et al*. (2000) contended that pasture production–weed interaction may have been detected accurately if weed dry matter rather than density is used in the analysis. This study concluded that neither DM production nor the density of *M. pudica* was a good indicator for predicting herbage production loss in tropical pastures because no significant relationship was found between *M. pudica* attributes and pasture herbage production (Tables 3 and 4).

CONCLUSION

Moderate grazing (LTG) system had adverse effect on *M. pudica* population; whereas heavy grazing (STG) supplied relatively desirable conditions for *M. pudica* establishment and infestation. Percent IV of *M. pudica* in LTG system was 46% lower than that in the grazing exclosure, whereas it was about three folds greater (220%) in the STG system than that in the ungrazed exclosure. DM production of *M. pudica* was generally similar between STG and ungrazed exclosure, whereas in the LTG system, it was 48% lower than that in the ungrazed exclosure. Proportion of *M. pudica* dry matter to pasture herbage production did not vary between grazed and ungrazed pastures in both LTG and STG systems. Neither DM production nor the density of *M. pudica* was a good indicator for predicting

herbage production loss in studied tropical pastures.

The findings of this study have a number of important implications for farmers and managers in tropical pastures. The intensity of grazing should not exceed moderate grazing of total biomass in tropical native and improved pastures in order to avoid loss in pasture productivity. The stocking density of 1 and 2.5–2.7 animal unit/ha can be appropriate cattle density per unit area in terms of minimal negative impacts on *M. pudica* invasion and maximum production in tropical native and improved pastures, respectively.

ACKNOWLEDGEMENTS

Funding for this project was provided by Universiti Putra Malaysia (UPM). The authors wish to thank the Department of Environmental Management of Universiti Putra Malaysia for facilitating the fieldwork.

REFERENCES

Chauhan, B. S., & Johnson, D. E. (2009a). Germination, emergence, and dormancy of Mimosa pudica. *Weed Biology and Management, 9,* 38−45.

Chauhan, B. S., & Johnson. D. E. (2009b). Influence of environmental factors on seed germination and seedling emergence of eclipta (*Eclipta prostrata*) in a tropical environment. *Weed Science*, *56,* 383–388.

Ferrell, J. A., Mullahey, J. J., Dusky, J. A., & Roka, F. M. (2006). Competition of giant smutgrass (*Sporobolus indicus*) in a bahiagrass pasture. *Weed Science, 54,* 100–105.

Frank, A. B., Tanaka, D. L., Hofmann, L., & Follett, R. F. (1995). Soil carbon and nitrogen of northern Great Plains grasslands as influenced by long term grazing. *Journal of Range Management*, *48,* 470–474.

Galinato, M. I., Moody, K., & Piggin, C. M. (1999). *Upland rice weeds of south and southeast Asia*. International Rice Research Institute, Makati, the Philippines.

Giliba, R. A., Boon, E. K., Kayombo, C. J., Musamba, E. B., Kashindye, A. M, & Shayo, P. F. (2011). Species composition, richness and diversity in Miombo woodland of Bereku Forest Reserve, Tanzania. *Journal of Biodiversity, 2*(1), 1–7.

Grekul, C. W., & Bork, E. W. (2004). Herbage yield losses in perennial pasture due to Canada Thistle (*Cirsium arvense*). *Weed Technology*, *18,* 784–794.

Harker, K. N., Baron, V. S., Chanasyk, D. S., Naeth, M. A., & Stevenson, F. C. (2000). Grazing intensity effects on weed populations in annual and perennial pasture systems. *Weed Science, 48,* 231-238.

Holm, L. G., Plucknett, D. L., Paucho, J. V. M., & Herberger, J. P. (1997). *The world's worst weeds, distribution and biology*. East-West Centre, University Press of Hawaii, Honolulu. 609 pp.

Hume, L. (1985). Crop losses in wheat (*Triticum aestivum*) as determined using weeded and non-weeded quadrats. *Weed Science, 33,* 734–740.

Kamaruzaman, J., & Nik, M. M. (1992). An analysis of soil disturbance from logging operation in a hill forest of Peninsular Malaysia. *Forest Ecology and Management, 47,* 323−333.

Li, C., Hao, X., Willms, W. D., Zhao, M., & Han, G. (2009). Seasonal response of herbage production and its nutrient and mineral contents to long-term cattle grazing on a rough fescue grassland. *Agriculture, Ecosystems and Environment, 132,* 32–38.

Liebig, M. A., Gross, J. R., Kronberg, S. L., Hanson, J. D., Frank, A. B., & Phillips, R. L. (2006). Soil

response to long term grazing in the northern Great Plains of North America. *Agriculture, Ecosystems and Environment, 115,* 270–276.

Lopez-Zamora, I., Comerford, N. B., & Muchovej, R. M. (2004). Root development and competitive ability of the invasive species *Melaleuca quinquenervia* (Cav.) S. T. Blake in the South Florida flatwoods. *Plant and Soil, 263,* 239–247.

MAFF. (Ministry of Agriculture, Fisheries, and Food) (1986). *The analysis of agricultural materials (3rd edn.).* Reference Book 427. Her Majesty's Stationery Office, London, United Kingdom.

Magda, D., Duru, M., Huguenin, J., & Gleizes, B. (2006). Impact of shading and cutting on the demography and composition of *Mimosa pudica* L., a ligneous weed species of tropical grasslands. *Grass and Forage Science*, *61,* 89–96.

Mannetje, L. (1978). *Measurement of grassland vegetation and animal production*. Commonwealth Agricultural Bureaux, Bulletin 52, Hurley. Berkshire, England.

Martınez, L. J., & Zinck, J. A. (2004). Temporal variation of soil compaction and deterioration of soil quality in pasture areas of Colombian Amazonia. *Soil and Tillage Research*, *75,* 3–17.

Mesdaghi, M. (2004). *Range Management in Iran*. Imam Reza University Press, Mashhad, Iran (In Persian).

Pragada, P. M., Padal, S. B., Krishna, B. R., Rao, D. S., & Narayana, V. (2011). Ecological aspects of weed flora of turmeric (*Curcuma longa* L.) fields of Visakhapatnam District, A.P., India. *Journal of Biodiversity and Environmental Sciences, 1*(6) 30-38.

Simonet, P. (1990). Sheep flock management in a tropical environment under coconut. *Oleagineux, 45,* 451–456.

Siregar, M. E., Haryanto, B., & Tjitrosemito, S. (1990). A review of weed management in Indonesian pastures. *BIOTROP*, *38,* 229–235.

SPSS Inc. (2007). *SPSS 16.0 for Windows, Release 16.0.1*. SPSS Inc. and IBM company, Chicago, Illinois.

Stohlgren, T. J., Bull, K. A., Otsuki, Y., Villa, C. A., & Lee, M. (1998). Riparian zones as havens for exotic plant species in the central grasslands. *Plant Ecology, 138,* 113–125.

Stur, W. W., & Shelton, H. M. (1990). Review of Forage Resources in Plantation Crops of Southeast Asia and the Pacific. *Forages for plantation crops workshop*. 27-29 June, Bali, Indonesia.

Tauseef, M., Ihsan, F., Nazir, W., & Farooq, J. (2012). Weed flora and importance value index (IVI) of the weeds in cotton crop fields in the region of Khanewal, Pakistan. *Pakistan Journal of Weed Science and Research*, *18,* 319–330.

Tracy, B. F., Renne, I. J., Gerrish, J., & Sanderson, M. A. (2004). Effects of plant diversity on invasion of weed species in experimental pasture communities. *Basic and Applied Ecology*, *5,* 543-50.

Tracy, B. F., & Sanderson, M. A. (2004). Forage productivity, species evenness and weed invasion in pasture communities. *Agriculture, Ecosystems and Environment, 102,* 175–183.

Van Der Wal, R., Egas, M., Van Der Veen, A., & Bakker, J. (2000). Effects of resource competition and herbivory on plant performance along a natural productivity gradient. *Journal of Ecology, 88,* 317–330.

Volkov, A. G., Foster, J. C., Ashby, T. A., Walker, R. K., Johnson, J. A., & Markin, V. S. (2010). *Mimosa pudica*: Electrical and mechanical stimulation of plant movements. *Plant, Cell and Environment, 33,* 163–173.

Wardle, D. A., Nicholson, K. S., & Rahman, A. (1995). Ecological effects of the invasive weed species *Senecio jacobaea* L. (ragwort) in a New Zealand pasture. *Agriculture, Ecosystems and Environment*, *56,* 19-28.

Wienhold, B. J., Hendrickson, J. R., & Karn, J. F. (2001). Pasture management influences on soil properties in the northern Great Plains. *Journal of Soil and Water Conservation, 56,* 27–31.

Yates, C. J., Norton, D. A., & Hobbs, R. J. (2000). Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-western Australia: implications for restoration. *Austral Ecology*, *25*, 36–47.

Pertanika J. Trop. Agric. Sci. 37 (2): 263 - 274 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Stored Carbon in Dominant Seaweeds of Indian Sundarbans

Mitra, A.[1,2]*, Zaman, S.[1,2], Pramanick, P.[1,2], Bhattacharyya, S. B.[1,2] and Raha, A. K.[1,2]

[1]Department of Marine Science, University of Calcutta, 35. Ballygunge Circular Road, Kolkata 700 019, West Bengal India

[2]Techno India University, Salt Lake Campus, Kolkata 700 091, West Bengal India

ABSTRACT

The stored carbon in the thallus of three seaweed species (*Enteromorpha intestinalis*, *Ulva lactuca* and *Catenella repens*) was estimated in three different seasons (pre-monsoon, monsoon and post-monsoon) from four sampling localities in the deltaic ecosystem of Indian Sundarbans ($21^0 40^/$ N to $22^0 40^/$N and $88^0 03^/$E to $89^0 07^/$E). The average stored carbon content varied from 1022.18 g m^{-2} (during monsoon) to 1067.02 g m^{-2} (during pre-monsoon) in *Enteromorpha intestinalis*. In *Ulva lactuca*, the stored carbon ranged from 46.41 g m^{-2} (during post-monsoon) to 152.43 g m^{-2} (during pre-monsoon). In case of *Catenella repens*, the range of stored carbon is 13.70 g m^{-2} (during monsoon) to 51.44 g m^{-2} (during pre-monsoon). Amongst the seaweed species undertaken in the study, *E. intestinalis* showed uniformity in biomass between sectors ($p_{cal} = 1.62 < p_{crit} = 18.51$) and seasons ($p_{cal} = 2.43 < p_{crit} = 4.14$), which implies tolerance of the species to variable salinity . Highest values of stored carbon were recorded in *E. intestinalis*, which might indicate its role as potential candidate species in the blue carbon series of Indian Sundarbans deltaic complex.

Keywords: Seaweeds, Standing stock, Carbon content, Indian Sundarbans

ARTICLE INFO

Article history:
Received: 20 May 2013
Accepted: 18 September 2013

E-mail addresses:
abhijit_mitra@hotmail.com (Mitra, A.),
sufia_zaman@yahoo.co.in (Zaman, S.),
ppramanick660@gmail.com (Pramanick, P.),
subhra_bikash@yahoo.com (Bhattacharyya, S. B.),
atanu_raha@hotmail.com (Raha, A. K.)
* Corresponding author

INTRODUCTION

The seas occupies 71% of the earth's surface and plays a dominant role in regulating climate, offering great potential for fixing and removing atmospheric carbon dioxide (Raven & Falkowski, 1999; Falkowski *et al*., 2000; Pelejero *et al*., 2010). Although macro-vegetated marine habitats account for 2% of the sea surface, they contribute ~210–244 Tg C $year^{-1}$ or ~50% of all carbon sequestered

ISSN: 1511-3701

in the global coastal oceans (Duarte *et al.,* 2005). The carbon storage potential of coastal marine vegetation (mangroves, salt marshes, and seagrass meadows) could be accelerated through various management approaches such as marine area protection, marine spatial planning, area-based fisheries management, regulated coastal development and ecosystem restoration (Laffoley & Grimsditch, 2009). This important floral community, often referred to as blue carbon, is cycled through food chains and metabolic processes in seas and oceans, where it becomes bound or sequestered in natural systems (Nellemann *et al.,* 2009). Seaweeds or benthic macroalgae, being important members in the blue carbon domain, are thallophytes containing photosynthetic pigments that live either in marine or brackishwater environs. Like their terrestrial counterpart, the seaweeds can prepare their own food with the help of sunlight and nutrient present in the seawater. They occupy the intertidal zones between high tide to low tide and up to a depth where 0.01 % photosynthetic light is available.

The primary productivity potential of seaweeds is quite high. Entrapment efficiencies of solar energy have been reported to be maximum in *Enteromorpha intestinalis* (0.64%) and *Ulva lactuca* (0.43%), with an average of 0.35% by this group. A research conducted on this topic indicates that in the deltaic complex of Indian Sundarbans, *E. intestinalis* and *U. lactuca* are the most productive species, followed by *E. prolifera* and *Rhizoclonium grande* (Chaudhuri & Choudhury, 1994).

Unlike other blue carbon sectors (mangroves, seagrasses and salt marshes), kelp forests and seaweed beds do not have such sedimentary substrata. Instead, their carbon-rich biomass detaches and is broken down in food chains by organisms that range in scale from grazing animals to pelagic and seabed bacteria. Knowledge on the scale of conversion of inorganic carbon into biomass, its subsequent sinking to the seabed and its sequestration over thousands of years form the basis of understanding the oceans as a potential sink for increasing levels of atmospheric carbon dioxide (CO_2). The other modes of fate of seaweed biomass depend on natural processes. The seaweed can be consumed by herbivores, whose faeces sink to the bottom and may remain there for a while. Moreover, distal portions of the fronds disintegrate during the summer season and those fragments enter the detritus food chain (Chung *et al*., 2013). Exudation as a dissolved organic material can be a critical loss. Therefore, some of the seaweed carbon will return to the water column and be either recaptured during photosynthesis or eventually returned to the atmosphere. However, a significant fraction of the algal carbon can be sequestered on the sea floor for a long period, perhaps centuries depending on location currents, etc. (Smetacek *et al.*, 2012).

Compared to other vegetation, the carbon sequestration potential of seaweeds in estuarine and deltaic environments, are however poorly understood. In this paper, the temporal and spatial variations in biomass production and carbon content

of three major species of seaweeds (*E. intestinalis, U. lactuca* and *C. repens*) inhabiting two different sectors of Indian Sundarbans (western and central) with contrasting salinity have been estimated. The results of the present investigation may serve as baseline data of stored carbon in the thallophytic community of this region.

MATERIALS AND METHODS

Sampling Site and Sample Collection

The Indian Sundarbans is a mangrove dominated deltaic complex situated at the confluence of the River Ganga and the Bay of Bengal. Two sampling sectors were selected each in and around the western and central sectors in the study region (see Fig.1). The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel in the late 15th century (Chaudhuri & Choudhury, 1994). Contrasting salinity thus exists in the deltaic complex that has made the region a unique test bed to observe the impact of salinity on biotic community. With this background, four sampling stations (two each in western and central sectors) were selected (Table 1 and Fig.1) to analyse the data of stored carbon in the common seaweed species.

Sampling

Seasonal samplings for biomass and carbon estimation of seaweed species (*E. intestinalis, U. lactuca and C. repens*) were carried out at ebb tides during May, 2012 (pre-monsoon), September, 2012 (monsoon) and December, 2012 (post-monsoon) during 2012 from the intertidal mudflats. Samples of seaweed species were scrapped and handpicked from sluice gates, mangrove trunk and concrete jetties. Immediately after collection, the thallus of each species was thoroughly washed separately in the ambient seawater, as well as with tap water, to remove adhering debris and sediments.

TABLE 1
Sampling stations with coordinates and salient features

Station	Coordinates (Lat and Long)	Salient Features
Nayachar Island (Stn. 1)	21° 45′ 24" N and 88° 15′ 24" E	It is located in the Hooghly estuary and faces the Haldia Port-cum-industrial complex that houses a variety of industrial units.
Sagar South (Stn. 2)	21° 39′ 04" N and 88° 01′ 47" E	Situated at the confluence of the River Hooghly and the Bay of Bengal on the western sector of Indian Sundarbans.
Gosaba (Stn. 3)	22° 15′ 45" N and 88° 39′ 46" E	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans.
Annpur in Satjelia Island (Stn. 4)	22° 11′ 52" N and 88° 50′ 43" E	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as control zone.

Altogether 10 quadrants (area, $1m^2$) were sampled for each species randomly mixed and weighed accurately in an electronic balance (IRD Balance; Model No. 290). The biomass is expressed in g m^{-2}.

Carbon Estimation

Seaweed samples were dried in a hot air oven (60ºC) for 72 hrs. (*www.academia.edu/755347/seagrasses_and_seaweeds*) until a constant weight was obtained. Dried

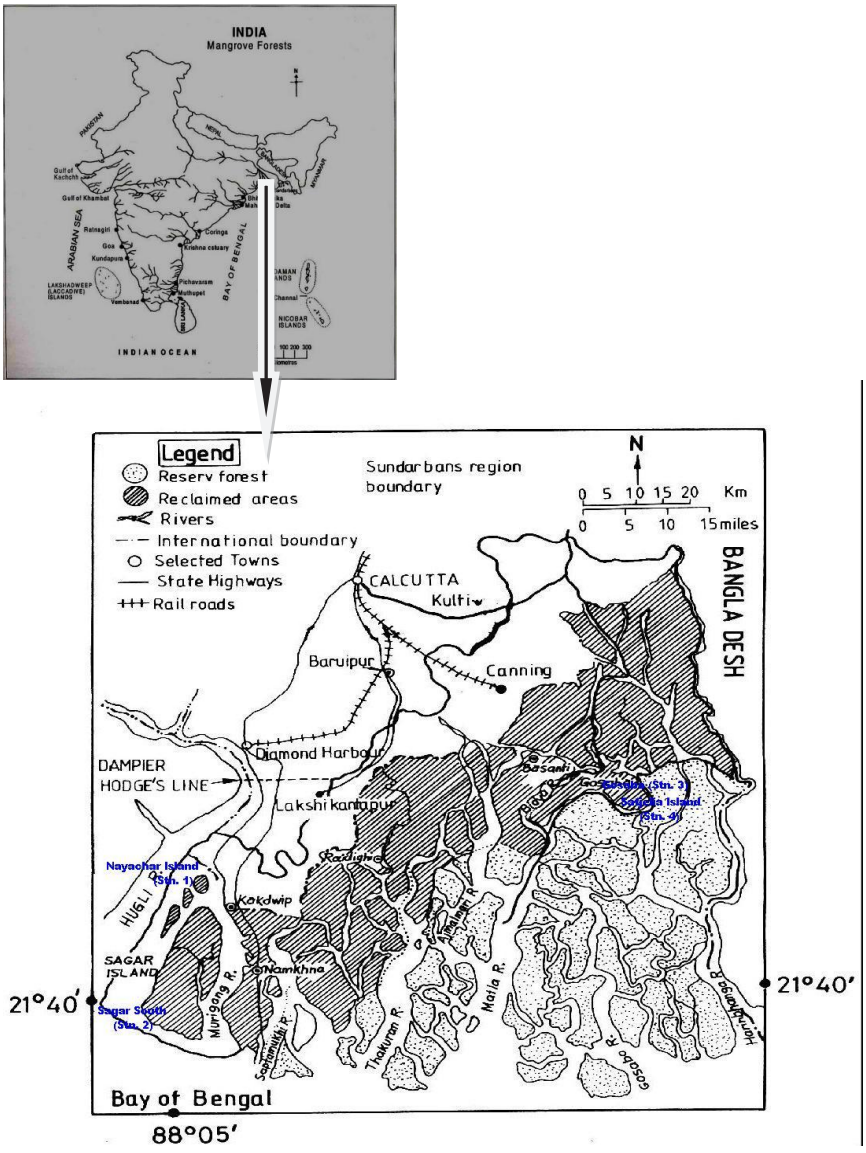


Fig.1. Location of the sampling stations (marked in blue) in Indian Sundarbans. Stns. 1 and 2 are in the western sector and Stns. 3 and 4 are in the central sector of the study area.

sampled were ground to a fine powder. Direct estimation of percent carbon in the thallus body of each seaweed species for each season and for each sampled locations was separately carried out by Vario MACRO elementar make CHN analyzer, after grinding and random mixing the oven dried seaweed samples. This method is followed for estimating carbon percentage in coastal vegetation (Mitra *et al*., 2011; Sengupta *et al*., 2013). At about 990^{o}C, the seaweed sample is mineralized. Formation of carbon monoxide is possible at this temperature even in the presence of excess oxygen. The complete oxidation is reached through a tungsten trioxide catalyst which is passed by the gaseous reaction products. The samples were finally analyzed through CHN mode, which is the most universal of the analysis mode because of the combination of the reagent design and the optimize combustion control parameters and expressed in percentage (Hedges & Stern, 1984). Calibration was done using 2, 5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (C% = 72.53; H% = 6.09%; N% = 6.51; O% = 7.43; S%= 7.44%) as calibration standard. Our observation did not show much deviation from the standard (for carbon = ±1.39%).

Statistical Analysis

Sector-wise and station-wise data on biomass and carbon content during different seasons in three seaweed species of Indian Sundarbans were subject to statistical analyses. Analysis of variance (ANOVA) expressed by Wellman (1998) was used to evaluate whether biomass and carbon content varied significantly between (i) stations (ii) the two sectors and (iii) seasons. Possibilities ($p < 0.01$) were considered statistically significant. All statistical calculations were performed with Statistical Package for Social Sciences (SPSS) 14.0 for Windows.

RESULTS AND DISCUSSION

The recent thrust on global warming phenomenon has generated tremendous interest in the carbon-storing ability of coastal vegetation. Carbon fixation by seaweeds forms an important bio-mechanism to diminish the increment of CO_2 in the atmosphere and thereby alleviate the trend toward global warming. Primary producers of coastal and marine biotopes such as microalgae, seaweed and seagrass are excellent carbon sequestering agents than their terrestrial counterparts (Zou, 2005). The carbon stored in the marine and estuarine floral species is referred to as blue carbon and a number of literatures have addressed the importance of the community to climate change (Nellemann *et al.,* 2009; Duarte *et al*., 2005). Several researches have been initiated on the carbon fixation capacity of seaweeds for the purpose of developing blue carbon register. One of the important problems in the sphere of blue carbon is the turnover time of the marine plants. According to Smith (1981), most of the terrestrial plants have a relatively high biomass and have a turnover time of several years to decades. On contrary, the turnover time of marine seaweeds is about one year

(Muraoka, 2004), although they have highest biomass among the marine ecosystems . This means that the seaweeds are more effective carbon sinks than phytoplankton, but less effective than the terrestrial ecosystem. The carbon sequestration in this unique producer community is a function of biomass production capacity, which in turn depends upon interaction between edaphic, climate and topographic factors of an area (Mitra *et al*., 2011; Jana *et al*., 2013). Hence, results obtained at one region may not be applicable to another. Therefore, region-based potential of storing and sequestering carbon by coastal vegetation on different land types or substratum characteristics needs to be estimated (Mitra *et al*., 2011). The seaweed carbon is acquired through photosynthetic and non-photosynthetic processes. Carbon assimilation in marine algae is largely accomplished by light dependent photosynthesis. However, there are active and significant light-independent carboxylation pathways operating as well (Cabello-Pasini, 1996). Cabello-Pasini and Alberte (2001) indicated that photosynthetic and non-photosynthtic carboxylation pathways are regulated, atleast partially, by the activity of Ribulose 1, 5 bis-phosphate carboxylase oxygenase (RUBISCO) and phosphoenolpyruvate carboxylase (PEPCK). Furthermore, differences between the *in vivo* and *in vitro* carboxylation in the thallus of *Laminaria setchellii* suggest structural, biochemical and functional differences that impact the dynamics of production of kelp species. Photosynthetic and light-independent carbon fixation (LICF) processes in marine algae have been shown to vary as a function of seasonal changes in irradiance and temperature, and carbohydrate levels in the tissue (Cabello-Pasini & Alberte, 1997). The seasonal variation of carbon content in seaweeds is attributed to variation in their biomass. The quantity of algal biomass that accumulates is normally stated as the amount of carbon fixed by photosynthesis per unit area of space or volume, per unit of time. Most estimates are expressed as net primary production, taking into account the costs of respiration (Chung *et al*., 2013).

Enteromorpha intestinalis

The biomass of *E. intestinalis* ranged from 2844.55 gm m^{-2} (at Stn. 3, during pre-monsoon 2012) to 3169.44 gm m^{-2} (at Stn. 1, during post-monsoon 2012). The carbon content exhibited lowest value at Stn. 3 (917.62 g m^{-2} during monsoon 2012) and highest at Stn. 2 (1141.67 g m^{-2} during pre-monsoon 2012) (Fig.2). It is interesting to note that the order of biomass of *E. intestinalis* is post-monsoon (3057.42 g m^{-2}) > monsoon (3008.08 g m^{-2}) > pre-monsoon (2967.29 g m^{-2}) (Table 2). However, the carbon content varied as per the order pre-monsoon (1067.02 g m^{-2}) > post-monsoon (1039.14 g m^{-2}) > monsoon (1022.18 g m^{-2}) (Table 3). The biomass of the species exhibited an almost uniform value in both the sectors and three seasons ($p < 0.01$) as revealed through ANOVA (Table 4), but the carbon content showed a significant

spatial and seasonal variations ($p < 0.01$) (Table 5). The highest carbon content in pre-monsoon may be attributed to congenial temperature and solar radiation in the study area that have positive influence on the photosynthetic rate.

Ulva lactuca

Fig.3 shows the seasonal variations of biomass and carbon content in *U. lactuca* that exhibit significant seasonal and spatial variations ($p < 0.01$) (Tables 4 and 5). The biomass ranged from 92.34 g m^{-2} (at Stn. 1, during monsoon 2012) to 786.52 g m^{-2} (at Stn. 2, during pre-monsoon 2012). The carbon content showed the lowest value at Stn. 1 during monsoon 2012 (25.11gm m^{-2}) and highest at Stn. 2 during pre-monsoon 2012 (245.55 g m^{-2}). In case of *U. lactuca* the order of biomass is pre-monsoon (495.84 g m^{-2}) > monsoon (249.12 g m^{-2}) >post-monsoon (160.49 g m^{-2}) (Table 2), whereas, the carbon content varied as per the order

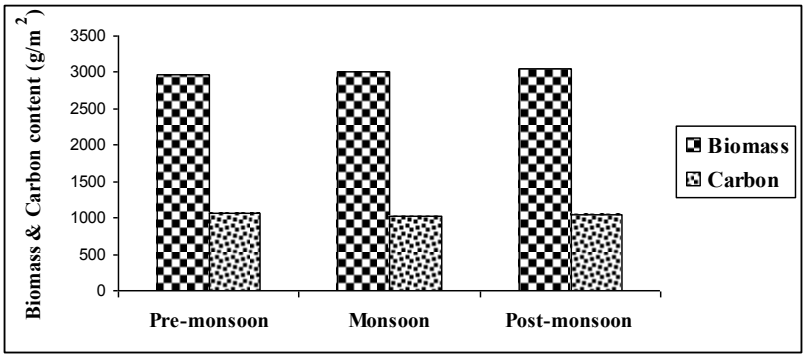


Fig.2: Seasonal variation in Biomass and Carbon content of *Enteromorpha intestinalis* during 2012

TABLE 2
Average seasonal variation in biomass (gm m^{-2}) of seaweeds with standard deviation

Species	Season		
	Pre-monsoon	Monsoon	Post-monsoon
Enteromorpha intestinalis	2967.29 ±26.85	3008.08 ±30.63	3057.42 ±33.25
Ulva lactuca	495.84 ±9.01	249.12 ±7.36	160.49 ±5.54
Catenella repens	229.94 ±4.52	66.47 ±2.81	132.0 ±1.73

TABLE 3
Average seasonal variation in carbon content (gm m^{-2}) of seaweeds with standard deviation

Species	Season		
	Pre-monsoon	Monsoon	Post-monsoon
Enteromorpha intestinalis	1067.02 ±2.85	1022.18 ±1.93	1039.14 ±2.03
Ulva lactuca	152.43 ±1.88	70.52 ±1.29	46.41 ±1.10
Catenella repens	51.44 ±0.93	13.70 ±0.70	27.58 ±0.62

pre-monsoon (152.43 g m^{-2}) > monsoon (70.52 g m^{-2}) > post-monsoon (46.41 g m^{-2}) (Table 3). The highest values of biomass and carbon in *U. lactuca* are again the result of synergistic effect of temperature and solar radiation.

Catenella repens

The biomass of *C. repens* collected from the selected stations ranged from 41.30 (at Stn. 1, during post-monsoon 2012) to 312.39 g m^{-2} (at Stn. 2, during pre-monsoon 2012). The order of biomass is pre-monsoon (229.94 g m^{-2}) > post-monsoon (132.00 g m^{-2}) > monsoon (66.47 g m^{-2}) for *C. repens* (Table 2). In the thallus body of the species, the values of stored carbon ranged from 8.03 (at Stn. 1, during post-monsoon 2012) to 74.51g m^{-2} (at Stn. 2, during pre-monsoon 2012) (Fig.4). The carbon content in this species varied as per the order pre-monsoon (51.44 g m^{-2}) > post-monsoon (27.58 g m^{-2}) > monsoon (13.70 g m^{-2}) (Table 3). ANOVA results (Table 4 and 5) also confirm significant spatial and seasonal variations in the biomass and carbon content of the species ($p < 0.01$).

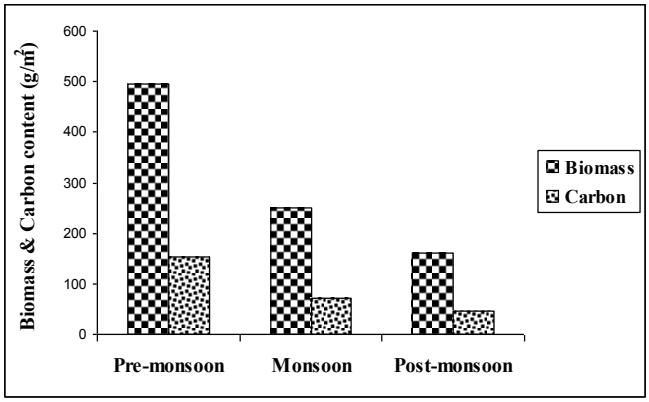


Fig.3: Seasonal variation in Biomass and Carbon content of *Ulva lactuca* during 2012

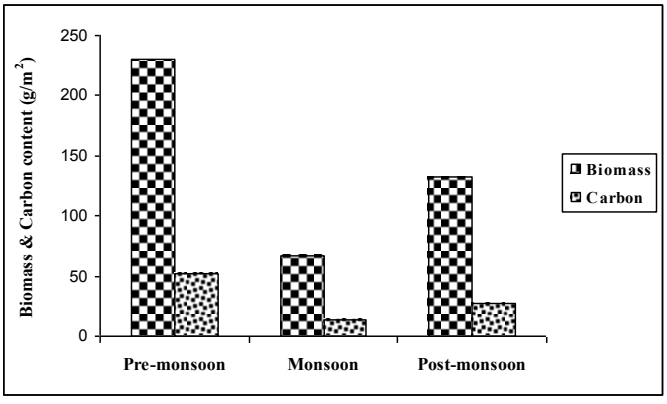


Fig.4. Seasonal variation in Biomass and Carbon content of *Catenella repens* during 2012

TABLE 4
Results of ANOVA for seaweed standing stock in Indian Sundarbans during (pre-monsoon, monsoon and post-monsoon seasons of 2102)

Variable	F_{cal}	F_{crit}
Enteromorpha intestinalis		
Between sectors	1.62	18.51
Between stations	7.77	4.76
Between seasons	2.43	4.14
Ulva lactuca		
Between sectors	1.12	18.51
Between stations	3.73	4.76
Between seasons	10.32	4.14
Catenella repens		
Between sectors	7.38	18.51
Between stations	6.89	4.76
Between seasons	14.70	4.14

TABLE 5
ANOVA for carbon content in seaweeds in unit area in Indian Sundarbans during 2102 (pre-monsoon, monsoon and post-monsoon seasons)

Variable	F_{cal}	F_{crit}
Enteromorpha intestinalis		
Between sectors	25.15	18.51
Between stations	38.31	4.76
Between seasons	4.64	4.14
Ulva lactuca		
Between sectors	0.84	18.51
Between stations	3.54	4.76
Between seasons	10.34	4.14
Catenella repens		
Between sectors	5.38	18.51
Between stations	5.86	4.76
Between seasons	13.23	4.14

The present study indicates that carbon storage in seaweed species is species-specific in nature. The highest value is observed in *E. intestinalis* (average 1042.77 g m^{-2}), followed by *U. lactuca* (average 89.79 g m^{-2}) and *C. repens* (average 32.64 g m^{-2}). Similar observation was also documented through a study done by Muraoka (2004), where the carbon absorption capacity by seaweeds varied as per the order *Laminaria* > *Ecklonia* > *Sargassum* > *Gelidium*. The species-wise variation of stored carbon may be attributed to the morphological structure of the seaweed. Unlike *U. lactuca* and *C. repens*, the extremely coiled and spiral structure of *E. intestinalis* exposes more area of the species to ambient water, which enables relatively more capture of carbon through diffusion. Due to presence of high surface area per unit volume of the *E. intestinalis* thallus, the absorption of carbon dioxide from the ambient water is more compared to *U. lactuca* and *C. repens*.

In the present study, *C. repens* is common seaweed under rhodophyceae which is characterized by reddish phycobilin pigments - phycoerythrin and phycocyanin- that mask the colour of the chlorophylls (the major photosynthetic machinery that synthesizes organic carbon through photosynthesis). This masking effect may be one of the reasons for lowest carbon content in *C. repens*.

Some interesting observations were documented with respect to biomass and stored carbon in the selected seaweeds species. These observations also point towards the tolerance of the species to ambient environment which is an important criterion for being a potential store house of carbon. In case of *E. intestinalis*, the ANOVA reflects no significant difference in biomass between sectors and seasons which shows a wide range of tolerance of this species to salinity (Table 2). It has also been documented that *E. intestinalis* can thrive

luxuriantly even in freshwater (Kamer & Fong, 2000). *U. lactuca* also exhibits almost similar trend with no statistically significant difference between sectors although the seasonal difference was observed. In case of *C. repens,* sector-wise differences in biomass were not discernible, but temporal variation was documented. A comparative study of biomass between three seaweeds species points out that *E. intestinalis* is one of the most potential species for carbon sequestration because of its uniform growth and biomass in different salinity regimes and seasons. Indian Sundarban is characterized with dynamic seasonal salinity profile with high value during pre-monsoon followed by monsoon and post-monsoon seasons. The spatial variation of salinity is also a unique feature of Indian Sundarbans (Mitra *et al.*, 2009; Mitra *et al.*, 2011; Sengupta *et al.*, 2013). In some areas of deltaic complex (particularly at station 1) the pre-monsoon salinity of ~ 10 psu drops down to 0 psu during monsoon (Mitra *et al.*, 2009). *E. intestinalis* can withstand such a drastic oscillation of salinity, and hence, can be a potential store house of carbon throughout the year (Fig.5). Except few experimental studies that observed the rate of absorption of CO_2, studies on seaweed carbon are scanty, particularly no baseline data are available on the carbon content in seaweed species collected from different salinity profile for comparative purposes. An experimental study conducted by Kaladharan *et al.* (2009) revealed that the green seaweed *Ulva lactuca* can register 100 % utilization of CO_2 towards carbon fixation from ambient water and beyond 15 mg/l there is a decline of 16%. The researchers also estimated that the seaweed biomass along the Indian coast is capable of utilizing

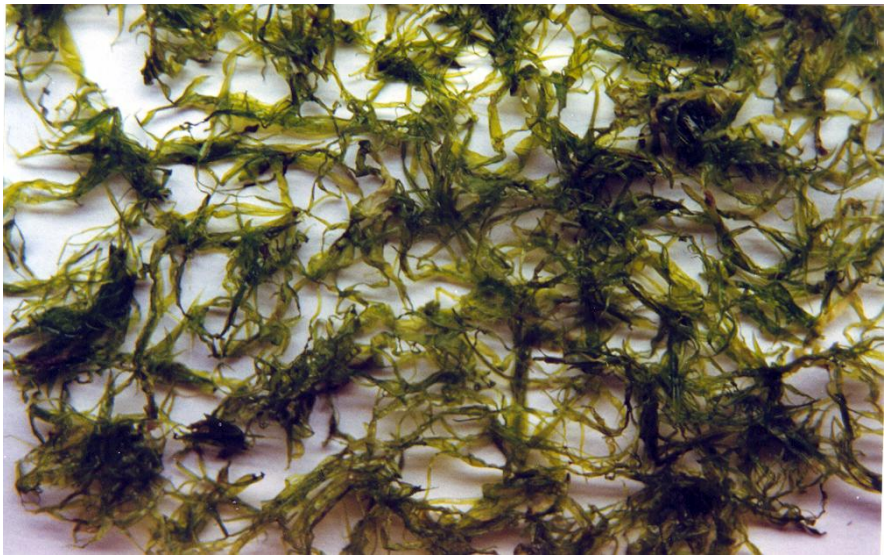


Fig.5: *E. intestinalis*: the most widely available seaweed species in Indian Sundarbans with highest carbon content

9052 t $CO_2 d^{-1}$ against emission of 365 t CO_2 d^{-1} indicating a net carbon credit of 8687 t d^{-1}.

The findings of the present research suggest that in the aquacultural sector, seaweed culture must be emphasized not only for their nutritional value, but also for their efficiency in storing carbon.

Due to the absence of thick sediment or soil layer beneath, seaweed beds obviously they lack functionality as large carbon sinks. It is, therefore, unlikely that the benefit of these marine resources can be addressed through carbon markets and management strategies that are strictly based on long term (centennial) sequestration. However, there is substantial potential to develop seaweed CDM methodologies by capturing carbon through algal photosynthesis and using the resulting biomass as a substitute for fossil hydrocarbons.

CONCLUSION

Seaweeds of Indian Sundarbans are potential store house of carbon. The storage efficiency, however, appears to be species-specific with highest carbon content in *Enteromorpha intestinalis*, followed by *Ulva lactuca* and *Catenella repens*. The stored carbon in the studied seaweed species also exhibits a distinct seasonal trend with highest values recorded during pre-monsoon. The uniformity of biomass of the *E. intestinalis* through seasons and sectors suggests that this species may tolerate wide fluctuations in the environmental variables. This characteristic is extremely important for a uniform carbon stock in a species throughout the year and requires a long term study to test and further validate the conclusion.

REFERENCES

Cabello-Pasini, A., & Alberte, S. R. (2001). Enzymatic regulation of photosynthetic and light-independent carbon fixation in *Laminaria setchellii* (Phaeophyta), *Ulva lactuca*, (Chlorophyta) and *Iridaea cordata* (Rhodophyta). *Revista chilena de historia natural*, *74* (2), ISSN 0716-078X; http://dx.doi.org/10.4067/S0716-078X2001000200002.

Cabello-Pasini, A. (1996). *Characterization and environmental regulation of light-independent carbon fixation in marine macrophytes*. (Ph.D. Dissetation). State University of New York, Stony Brook, New York. 322 pp.

Cabello-Pasini, A., & Alberte, R. S. (1997). Seasonal patterns of photosynthesis and light-independent carbon fixation in marine macrophytes. *Journal of Phycology, 33*, 321-329.

Chaudhuri, A. B., & Choudhury, A. (1994). *Mangroves of the Sundarbans*, India. 1, Bangkok, Thailand: IUCN.

Chung, I. K., Oak, J. H., Lee, J. A., Shin, J. A., Kim, J. G., & Park, K-S. (2013). Installing Kelp Forests/ seaweed beds for mitigation and adaptation against global warming: Korean project Review. *ICES Journal of Marine Science*, doi. 10.1093/ icesjms/fss206.

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences*, *2,* 1–8.

Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., & Gruber, N. (2000). The global carbon cycle: a test of our knowledge of Earth as a system. *Science*, *290*, 291–296.

Hedges, J. I., & Stern, J. H. (1984). Carbon and Nitrogen determinations of carbonate-containing solids. *Limnological Oceanography*, *29*(3), 657-663.

Jana, H., Zaman, S., Chakraborty, S., Pramanick, P., Mondal, K. C., & Mitra, A. (2013). Spatio-temporal variation of stored carbon in *Porteresia coarctata* along the East and West Coast of India. *International Journal of Engineering and Management Sciences, 4*(3), 377-381.

Kaladharan, P., Veena, S., & Vivekanandan, E. (2009). Carbon sequestration by a few marine algae: observation and projection. *Journal of Marine Biological Association of India*, *51*(1), 107-110.

Kamer, K., & fong, P. (2000). A fluctuating salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalgae, *Enteromorpha intestinalis* (L.) Link. *Journal of Experimental Marine Biology Ecology*, *254*(1), 53-69.

Laffoley, D. d'A., & Grimsditch, G. (2009). *The Management of Natural Coastal Carbon Sinks. IUCN, Gland, Switzerland.* 53 pp.

Mitra, A., Chowdhury, R., & Banerjee, K. (2011). Concentrations of some heavy metals in commercially important finfish and shellfish of the River Ganga. *Environmental Monitoring and Assessment, 184*, 2219-2230, doi: 10.1007/s10661-011-2111-x.

Mitra, A., Gangopadhyay, A., Dube, A., Schmidt, A. C. K., & Banerjee, K. (2009). Observed changes in water mass properties in the Indian Sundarbans (northwestern Bay of Bengal) during 1980–2007. *Current Science, 97*, 1445-1452.

Mitra, A., Sengupta, K., & Banerjee, K. (2011). Standing biomass and carbon storage of above-ground structures in dominant mangrove trees in the Sundarbans. *Forest Ecology and Management (ELSEVIER DOI:10.1016/j.foreco.2011.01.012)*, *261(7)*, 1325 -1335.

Muraoka, D. (2004). Seaweed resources as a source of Carbon fixation. *Bulletin Fisheries Research Agency,* Supplement *1*, 59-63.

Nellemann, C., Corcoran, E., Duarte, C. M., Valde´s, L., De Young, C., Fonseca, L., & Grimsditch, G. (Eds.) (2009). Blue Carbon: The role of healthy oceans in binding carbon. *A Rapid Response Assessment. Grid-Arendal/UNEP Arenda.* 78 pp.

Pelejero, C., Calv., E., & Hoegh-Guldberg, O. (2010). Paleo perspectives on ocean acidification. *Trends in Ecology and Evolution*, *25*, 332–344.

Raven, J. A., & Falkowski, P. G. (1999). Oceanic sinks for atmospheric CO_2. *Plant, Cell and Environment*, *22*, 741–755.

Sengupta, K., Roy Choudhury, M., Bhattacharya, S. B., Raha, A., Zaman, S., & Mitra, A. (2013). Spatial variation of stored carbon in *Avicennia alba* of Indian Sundarbans. *Discovery Nature*, *3*(8), 19-24.

Siegenthaler, U., & Sarmiento, J. L. (1993). Atmospheric carbon dioxide and the ocean. *Nature*, *365*, 119-125.

Smetacek, V., Klass, C., Strass, V. H., Assmy, P., Montressor, M., Cisewski, B., & Savoye, N. (2012). Deep carbon export from a southern ocean iron-fertilized diatom bloom. *Nature*, *487*, 313-319.

Smith, S. V. (1981). Marine macrophytes as a global carbon sink. *Science*, *211,* 838–840.

Wellman, B. (1998). Doing it ourselves: The SPSS Manual as Sociology's Most Influential Recent Book. In D. Clauson (Ed.), *Required Reading: Sociology's Most Influential Book* (pp. 71-78). Amherst: University of Massachusetts Press.

Zou, D. (2005). Effects of elevated atmospheric CO_2 growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). *Aquaculture*, *250*, 726-735.

Pertanika J. Trop. Agric. Sci. 37 (2): 275 - 283 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Mixed Viral Infection and Growth Stage on Chilli (*Capsicum annuum* L.) Production

Nurhayati Damiri

Department of Plant Pests and Diseases, Faculty of Agriculture, Sriwijaya University, Jalan Raya Palembang-Prabumulih, km 32, Ogan Ilir, Inderalaya 30662, South Sumatra, Indonesia

ABSTRACT

The objective of this research was to study the effects of mixed viral infection and growth stage on chilli (*Capsicum annuum* L.) production. This study was carried out in a split plot design with plant stage as the main plot and viral infection as the sub-plot. Plant stage as the main plot consisted of four levels, i.e 15 days, after transplanting (DAT), 40 DAT, 65 DAT, and 90 DAT, whereas viral infection as sub-plot consisted of 5 innoculation of viruses, i.e., no viral infection (control), cucumber mosaic virus (CMV) + tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) + potato virus Y (PYV), CMV+PYV and CMV+PYV+TMV. Each treatment was replicated five times. The inoculations were conducted mechanically by rubbing single young leaves which had been dusted with carborandum 400 mesh, with inoculum of respective viruses. Parameters observed in this research were plant height, biomass, and chillies production (number of fruits and the weight of total fruits yield) for each plant. Results of the study showed that that growth stage and viruses significantly affect the plant height and yield components of chilli. Mixed viral infection among CMV, PYV and TMV caused a significant reduction in the chilli biomass and production. Although viral infection increased the plant height, the infected chilli seemed unhealthy. There existed interaction effects of mixed viral infection and growth stages on the chilli biomass. All viral infection and growth stages reduced significantly the biomass of the chilli, with the lowest found at the mixed viral infection of TMV+PYV (18.5%) and the highest was at CMV+TMV (44%). Double mixed infection of CMV+TMV and CMV+PYV caused 52 and 49% reduction of both the total number of fruits and total weight of fruits/plant respectively being the highest reduction compared to other treatments.

Keywords: Chilli production, tobacco mosaic virus, potato virus Y, cucumber mosaic virus, growth stage

ARTICLE INFO

Article history:
Received: 10 June 2013
Accepted: 3 October 2013

E-mail address:
nurhayatidamiri@yahoo.co.id (Nurhayati Damiri)

ISSN: 1511-3701

INTRODUCTION

Chilli (*Capsicum annuum* L.) production in Indonesia cannot fulfil the national needs for chilli, forcing the Government of Indonesia to import chilli of up to 16,000 tonnes per annum (MOA, 2009). On average, chilli production in Indonesia is 4.35 tonnes per hectare, the amount which is far from potential production, that is, 10 tons per hectare. One constraint hampering the chilli production in Indonesia and other countries is viral disease (Suryaningsih *et al*., 1996). In Indonesia, under certain condition the infection of viral diseases can reach more than 90 percent of the chilli production area (Duriat, 1996).

Viral diseases on chilli are considered as the main limiting factor in the chilli cultivation, especially in Indonesia. In Asia, at least 35 viruses are known to infect the chilli plantation and the most important viruses are cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), potato virus Y (PYV). Mixed infection of the viruses can cause more severe diseases on the pepper plant (Arogundade *et al.*, 2012). At least ten different viruses have been reported to infect a number of chilli cultivars in Indonesia (Duriat, 1996; Suryaningsih, Sutarya, & Duriat, 1996). Four of them are cucumber mosaic virus (CMV), chilli veinal mottle virus (ChiVMV), potato virus Y (PYV) and tobacco mosaic virus (TMV), all of which can induce mosaic symptoms (Nurdin, 1998; Sulandari, 2004). Virus diseases caused yield loss in the range of 60-100% and these were considered the major constraints to the economic production of the crop (Green, 1993; Subekti, 2005).

Experts have noted that there exist around 13-35 viruses which attack chilli plants in the chilli plantation of tropical and sub-tropical regions. Prevalence of viral diseases from time to time have changed, as shown by the research results of Researh Centre for Vegetable Crops in Lembang Bandung West Java, Indonesia, between 1995 and 1996 (Duriat & Gunaini, 2003). Taufik *et al*. (2005) stated that CMV, PYV and TMV have spread throughout most of the chilli crops in Indonesia. A very recent study in South Sulawesi by Taufik *et al*. (2011) reported that CMV, together with TMV, infects pepper plants.

The objective of this research was to study the effects of mixed infection between TMV, CMV and PYV and growth stage on chilli production. Such information is very important in determining the proper means of controlling the diseases caused by viruses in the chilli production.

MATERIALS AND METHODS

This research was carried out in a green house at Bukit Lama Palembang Indonesia between January and August 2011 using a split plot design with plant stage as the main plot and viral infection as the sub-plot. Plant stage as main plot consisted of four levels, i.e. 15 days after transplanting (DAT), 40 DAT, 65 DAT, and 90 DAT, whereas viral infection as sub-plot consisted of 5 innoculation of viruses, i.e. no viral infection (control), CMV+TMV, CMV+PYV, CMV+PYV

and CMV+PYV+TMV. Each treatment was replicated five times. Local hot chilli seedling (15 days old) were transplanted onto polybags containing 10 kg of sterile soil mix (top soil:organic soil = 2:1). The inoculations were conducted mechanically by rubbing single young leaves which had been dusted with carborandum 400 mesh, with inoculum of respective viruses.

The parameters observed in this study were plant height, biomass, and chillies production (number of fruits and the weight of total fruits yield) by the each plant. The data were analyzed to determine the effects of virus on vegetative growth and yield using the analysis of variance (ANOVA), with the Duncan's Multiple Range Test (DMRT) comparison among means (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Analysis result of the effects of the overall treatments in the study showed that growth stage and viruses singly significantly affected the plant height and yield components of chilli. The interaction effect of the chilli growth stage and viral infection significantly affected the plant height, average weight of fruit and biomass of the tested chilli plants. However, there was no interaction effect of growth stage and viral infection on the number of fruits and weight of fruits (Table 1).

The results of further test showed that the growth stage at the time of viral inoculation significantly affected the plant height (Table 2). Based on the test for a number of viral inoculation on the four growth stages 15 DAT, 40 DAT and 90 DAT, there existed a number of viral treatment which increased significantly the plant height. Relative values (percentage towards control) of the plant height at 15 DAT ranging from 78.9-119.7%, compared with the relative values of 40 DAT (98.5-149.3%) and 90 DAT (105.3-132.9%).

In Table 2, it was clear that CMV+TMV resulted in the highest plant heights of chilli at 15 and 40 DAT, while at 90 DAT the highest plant height was at CMV+PYV+TMV. However, all inoculated chilli plants in this study, which had relatively higher plant height seemed to be unhealthy compared to the control (see Fig.1). This result is

TABLE 1
Summary of the effects of viral infection and growth stage on the plant height and chilli production

No	Observed variables	Treatment		
		Growth stage	Viral infection	growth stage*Virus
1	Plant height	**	**	**
2	Number of fruits	**	**	ns
3	Weight of fruits	**	**	ns
4	Average weight of fruit	**	**	*
5	Biomass	**	**	*

** and * = significantly different at $p < 0.01$ and ≤ 0.05, respectively
ns = not significantly different

not surprising for other reseachers such as Kusumawati, Hadiastono and Martosudiro (2013) who reported that viral attack such as TMV on various growth stage can suppress plant growth and decrease cayenne pepper production.

Further test (Table 3) showed that there existed interaction effects of mixed viral infection and growth stages on the chilli biomass. All double and triple viral infection significantly reduced biomass regardless of the growth stage of the chilli plant. Relative values of the biomass at 15 DAT had reduction ranging from 64.9-81.5%, followed by those of 40 DAT ranging from 65.2-71.5%, and those of 65 DAT ranging from 60-64.1% compared with control (no viral treatment). Surprisingly, relative values of biomass at 90 DAT are highest which is ranging from 46.1-78% compared with the control. The lowest reduction of chilli biomass was at the mixed viral infection of TMV+PYV (18.5%) and the highest was at CMV+TMV (44%). Reduction of 58% of cayenne pepper biomass due to viral attact by TMV was reported by Kusumawati, Hadiastono and Martosudiro (2013). Meanwhile, Gallitelli (1998) and Suryaningrat *et al*. (1996) have reported the presence of CMV, together with other viruses in chilli plantation, which has caused big losses in chili yield.

The amount of reduction in chilli production in the study was clearly seen in further test. The results of the test (Table 4) showed that the effect of mixed viral infection among CMV, TMV, PYV caused a significant reduction in the chilli production. Double mixed infection of CMV+TMV and CMV+PYV caused 52 and 49% reductions of both the total number of fruits and total weight of fruits/plant, respectively. These

Fig.1: Infection symptoms of the viruses attack on leaves at 90 DAT
(from leaf to right – control, CMV+TMV, CMV+PYV, TMV+PYV, CMV+TMV+PYV)

were also found as the highest reductions compared to the other treatments. The mixed infection of plant viruses that has caused severe symptoms showed that interaction among viruses occurs within plant tissues (Kosaka & Funishi, 1997; Zhang *et al*., 2001). The interaction between two or more of virus pathogen can occur either synergistic, additive or antagonism (Oku, 1994; Matthew, 1991). A study by Akin and Nurdin (2003) reported a reduction in their chilli yield ranging 21-67% due to TMV attack. They also added that TMV inhibitted generative growth in the chilli crop.

In Table 4, the growth stage is shown to cause significant reduction in chilli yields, i.e. number of fruits per plant and total weight of fruits per plant. The growth stages of 15 DAT, 65 DAT and 90 DAT reduced significantly the number of fruits per plant, which ranged from 42 to 38% compared to the control. Gowth stage also reduced significantly the total weight of fruits per plant. Growth stage of 65 DAT, followed by 90 DAT and 15 DAT caused reductions in the total weight of fruits per plant, which ranged from 46 to 31.5% compared to the control. A study conducted by Kusumawati,

TABLE 2
The interaction effects of mixed viral Inoculations and growth stages of the chilli on plant height (cm)

	Days after transplanting (DAT)											
Viral treatment	15			40			65			90		
No virus	71		ab	69		a	79		a	85		a
CMV+TMV	56	(78.9)	a	72	(104.3)	a	71	(89.9)	a	89.5	(105.3)	a
CMV+PYV	85	(119.7)	b	103	(149.3)	b	77	(97.5)	a	100	(117.6)	ab
TMV+PYV	59	(83)	a	82	(118.8)	a	71	(89.9)	a	91	(107.6)	a
CMV+PYV+TMV	65.5	(92.2)	a	68	(98.5)	a	65	(82.3)	a	113	(132.9)	b
Average	67.3	(93.7)		78.8	(117.8)		72.6	(89.9)		95.7	(115.85)	

Figures in a column that are followed with the same letters mean there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

TABLE 3
The interaction effects of mixed viral inoculations and growth stages of the chilli on biomass (g)

	Days after transplanting (DAT)											
Viral treatment	15			40			65			90		
No virus	157		b	214		b	170		b	178		c
CMV+TMV	108	(68.8)	a	139.5	(65.2)	a	108	(63.5)	a	82	(46.1)	a
CMV+PYV	102	(64.9)	a	153	(71.5)	a	109	(64.1)	a	139	(78)	ab
TMV+PYV	128	(81.5)	a	136	(63.5)	a	102	(60)	a	84	(47.2)	a
CMV+PYV+TMV	119	(75.8)	a	134	(62.6)	a	102	(60)	a	133	(74.7)	b
Average	122.8	(72.72)		155.3	(65.7)		118	(61.9)		103	(61.5)	

Figures in a column that are followed by the same letters mean that there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

Hadiastono and Martosudiro (2013) was in line with this study's findings, whereby growth stage at the time of viral inoculation reduced cayenne pepper significantly.

An interaction effect of viral infection and growth stage was found on the average weight of fruit. Mixed viral infection of CMV+PYV at 15 DAT reduced the average weight of fruit around 30% compared to the control. In contrast, the mixed viral infection of CMV+TMV, CMV+PYV and TMV+PYV increased the average weight of the chilli fruit. This is not surprising for the chilli's total number of fruits per plant and total weight of fruits per plant for the treatments, which were lower than the control. It is believed that the rotten fruits are due to viruses containing more water compared to healthier plants (the control) (see Table 5). Aeni (2007) reported that the

TABLE 4
The effects of mixed viral innoculations and growth stages on chilli production

Factors tested	Total number of fruits per plants			Total weight of fruits/plant (g)		
Viral treatment						
No virus	53		b	238		c
CMV+TMV	24	(48)	a	115	(48.3)	a
CMV+PYV	25.5	(51)	a	123	(51.7)	a
CMV+PYV+TMV	43	(81)	a	132	(55.5)	ab
PYV+TMV	53	(100)	b	173	(72.7)	b
Growth stage						
15 DAT	33	(62)	a	163	(68.5)	ab
40 DAT	47	(88.7)	b	179.5	(75.4)	b
65 DAT	29	(54.7)	a	129	(54)	a
90 DAT	28	(52.8)	a	143	(60)	ab

Figures in a column that are followed by the same letters mean that there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

TABLE 5
The interaction effects of mixed viral inoculations and growth stages of the chilli on the average weight of fruit (g)

	Days after transplanting (DAT)											
Viral treatment	15			40			65			90		
No virus	5.1		b	4		ab	4.8		a	4.4		a
CMV+TMV	5.3	(104)	b	3.5	(90)	a	4.7	(97.9)	a	6.6	(150)	b
CMV+PYV	3.8	(70)	a	5.3	(132)	b	5.3	(110.4)	a	5.8	(132)	b
TMV+PYV	4.8	(130)	ab	3.4	(85)	a	4	(83.3)	a	6.2	(140)	b
CMV+PYV+TMV	5.3	(110)	b	3.8	(110)	a	4.5	(93.7)	a	5.5	(129.5)	ab
Average	3.86	(110)		4	(125.5)		4.6	(96.3)		5.7	(137.9)	

Figures in a column that are followed by the same letters mean there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

nett assimilation of the affected chilli plants by viruses was much lower than that of the healthy ones causing lower production of the chilli crop.

The low chilli production from the treatment of mixed TMV with other viruses is clearly related to the attack symptom shown by every virus or their combination (Sutarya, 1991). The yield reduction happened to all viral treatments. However, the highest reduction was for the CMV+PYV treatment, followed by other mixed viral treatments. The least resistance of chilli growth stage was by the time chilli having its first flowers. At this stage, the chilli plants are in need of more nutrition on the one hand, and experiencing metabolism disturbance on the other. In this situation, the plants suffered heavy stress. The plants that were attacked by viruses experienced heavy chlorosis in their leaves as the viruses disturb the enzym anaplerotic, which lower the rate of the plants photosynthesis rate (Fanayama & Terashima, 2006). This is in agreement with a report by Goodman, Kiraly and Zaitin (1967). Akin and Nurdin (2003), Gallitelli (1998), and Suryaningsi *et al*. (1996) have reported big losses in chilli yields as a result of CMV and TMV attacks in their chilli crops.

CONCLUSION

From this research, it can be concluded that growth stage and viruses significantly affect the plant height and yield components of chilli. In specific, the mixed viral infection among CMV, PYV, and TMV caused significant reductions in the biomass and chilli production. Although viral infection increased the plant height, the infected chilli seemed unhealthy. The interaction effects of mixed viral infection and growth stages were found on the chilli biomass. All viral infection and growth stages reduced significantly the biomass of the chilli, with the lowest reduction at the mixed viral infection of TMV+PYV (18.5%) and the highest at CMV+TMV (44%). Double mixed infection of CMV+TMV and CMV+PYV caused 52 and 49% reductions of both the total number of fruits and total weight of fruits/plant, respectively, being the highest reductions compared to the other treatments.

REFERENCES

Aeni, N. A. (2007). *Study the stability of the production of chili curly yellow virus in endemic areas with optimization of plant nutrition*. (PhD Dissertation). Postgraduate Programme, Gajah Mada University of Yogyakarta, Indonesia.

Akin, H. M., & Nurdin, M. (2003). Effect of TMV (*Tobacco Mosaic Virus*) infection on the vegetative and generative growth of several varities of red chilli. *Journal of Tropical Pest and Disease, 3*(1), 10-12.

Arogundale, O., Bologun, S., & Kareem, K. T. (2012). Occurance and distribution of pepper veinal mottle and Cucumber mosaic virus in pepper in Ibadan, Nigeria. *J. Virology, 9*, 79. Doi:10.1186/1743-422x-9-79.

Duriat, A. S. (1996). Management of pepper viruses in Indonesia. *Problem and progress IARDJ, 18*, 45-50.

Duriat, A. S., & Gunaini. (2003). *Introduction to viral disease on chilli and its control.* Research Institute for vegetables. Body for research and

development in agriculture, Lembang, Bandung Indonesia.

Fumayama, D., & Terashima, I. (2006). Effect of Euphatorium yellow vein virus infection on photosynthetic rate, chlorophyll content and chloroplast structure in leaves of Euphatorium makinoi during leaf development. *Functional Plant Biology*, 165-175.

Gallitelli, D. (1998). Present status of controlling cucumber mosaic virus (CMV). In A. Hadidi, R.K. Khitarpal, & H. Koganezawa. (Eds.), *Plant virus disease control* (pp. 507-523). APS Press.

Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research (2nd edn.). An international rice research institute book.* New York-Chichester-Brisbane-Toronto-Singapore: A Wiley-Intersci. Publ., John Wiley and Sons.

Goodman, R., Kiraly, N. Z., & Zaitlin, M. (1967). *Biochemistry and physiology of infection plant disease*. London: O. Van. Nastrand Company. Inc.

Green, K. S. (1993). Pepper virus Research in Taiwan and other Asian country. Proceeding of the symposium on plant virus and virus-like disease. p 213-143. Council of Agricultural Plant Protection Series No. 1.

Indonesian Ministry of Agriculture. (2009). *Harvesting area and Production of Horticultural plants in Indonesia*. Jakarta.

Kosaka, Y., & Fukunishi, T. (1997). Multiple inoculation with three attenuated viruses for control of cucumber virus disease. *Plant Disease, 81*, 733-738.

Kusumawati, D. E., Hadiastono, T., & Martosudiro, M. (2013). Resistance of five cayenne pepper cultivars (Capsicum frutescens L.) on TMV (Tobacco Mosaic Virus) infection at different plant growth stages. *Journal of Plant Pests and Diseases, 1*(1), 66-79.

Matthew, R. E. F. (1992). Fundamentals of plant virology. San Diego: Academic Press.

Nurdin. (1998). *Identification of mosaic and curl causes viruses on Chilli* (*Capsicum annum* L.). Thesis of Post Graduate Studies, Bogor Agricultural University, Bogor, West Java Indonesia.

Oku, H. (1994). *Plant Pathogenesis and disease control*. Boca Raton: Lewis Publisher.

Subekti, D. (2005). *Evaluation of five chilli cultivars resistance towards the infection of Cucumber mosaic cucumovirus and Chilli vein mottle potyvirus: effects of single and mix infection on chilli yield*. Tesis of Postgraduate studies of Bogor University of Agriculture.

Sulandari, J. (2004). *Characterization biology, serology and DNA fingerprint analysis of disease-causing viruses dry leaves yellow peppers*. Dissertation in Graduate Programme, Bogor Agricultural University. Bogor. Indonesia.

Suryaningsih, R., Sutarya, & Duriat, A. S. (1996). Red chilli diseases and their control. In A. S. Duriat, W. Wijaya, A. Hadisoeganda, T. A. Soetiarso, & L. Prabaningum (Eds.), *Production Technology for Red Chilli* (pp. 64-84). Center for Research and Development of Horticulture, Research Institute and Agricultural Development.

Sutarya, R. (1991). Effect of CMV infection on the growth of spinach (*Amaranthus* spp.). *Bulletin of Research in Horticulture, XXI*, 1/1991.

Taufik, M., Hidayat, G., Suastika, S., Sumaraw, M., & Sugiprihati, S. (2005). Study of plant growth promoting Rhizobacteria as a potential agent of cucumber mosaic virus and chili vernal mottle virus in pepper. *Hayati Journal, 12*, 139-144.

Taufik, K., Khairuni, A., & Rambe, W. S. L. (2011). Use of ELISA for the detection of cucumber mosaic virus and tobacco mosaic virus in pepper. *Fitomedika Journal, 7*(3), 195-200.

Zhang, X. S., Holt, J., & Colvin, J. (2001). Sinergism between plant viruses: a mathematical analyses of the epidemiological implications. *Plant Pathol*., *50*, 735-746.

Pertanika J. Trop. Agric. Sci. 37 (2): 285 - 291 (2014)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Determination of *Pediobius* sp. (Hymenoptera: Eulophidae), A New Species Record of Endoparasitoid Associate with Beet Armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidea) from Malaysia using DNA Barcode

Ghazali, S. Z., Md-Zain, B. M. and Yaakop, S.*

School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

ABSTRACT

Spodoptera exigua (Lepidoptera: Noctuidea) is a polyphagous pest that attacks many important agricultural crops. Identifying an insect specimen is a crucial step in entomology. This study demonstrated a molecular method to identify the species of pest and its parasitoid in the case of a lack of a morphological identification key. To facilitate the identification of these pest-parasitoid species, a DNA barcoding approach was used for accurate and time-consuming identification based on nucleotide sequencing analysis of the mitochondrial *Cytochrome Oxidase subunit I* (*COI*) gene. DNA barcoding sequences successfully identified both pest and parasitoid species by comparing barcode sequences to the GenBank database. This study provided evidence of *Pediobius* sp. as a parasitoid of *S. exigua* in Malaysia.

Keywords: DNA barcoding, COI gene, *Spodoptera exigua*, *Pediobius* sp., fern

INTRODUCTION

Beet Armyworm or Small Mottled Willow Moth, *Spodoptera exigua* (Lepidoptera: Noctuidea) is a highly polyphagous pest that attacks many important agricultural crops (Ruberson *et al*., 1994) and damages many crop species worldwide (Hassanein *et al*., 1972; Aarvik, 1981; Stewart *et al*., 1996; Tisdale, 2001). However, its origin still remains unclear although it appears to be native to southern Asia (Ruberson *et al*., 1994). This moth species was first reported to infest asparagus fern, gladiolus and grasses (Ruberson *et al*., 1994) in the United States in 1876 (Harvey, 1876) and then dispersed into Florida in the late 1920s.

ARTICLE INFO

Article history:
Received: 25 June 2013
Accepted: 27 November 2013

E-mail addresses:
zafirah_najah@yahoo.com (Ghazali, S. Z.),
abgbadd@ukm.my (Md-Zain, B. M.),
salmah78@ukm.my (Yaakop, S.)
* Corresponding author

ISSN: 1511-3701

Beet armyworm has now become a serious pest that attacks various hosts ranging from economically important crops such as corn, cotton, soybean, peanuts, cabbage, tomatoes, to peppers (Pearson, 1982). Ruberson *et al*. (1994) listed a large number of parasitoid and predators of beet armyworm eggs and larvae predominantly from the families Hymenoptera, Braconidae and Ichneumonidae. In Malaysia, two parasitoid species of *S. exigua*, namely, *Microplitis manilae* Ashmead (Hymenoptera: Braconidae) and *Peribaea orbata* (Wiedemann) (Diptera**:** Tachinidae) have been reported by Sivapragasam and Syed (2001) and Sivapragasam *et al*. (2001). The two species are parasitoids to the larval stages of *S. exigua* and both species have been considered major larval parasitoids of *S. exigua* in Malaysia (Azidah, 2007).

Molecular methods are now widely used compared to conventional methods. Conventional approaches were performed by host-parasitoid rearing and host dissection (Day, 1994). These approaches have many disadvantages because during the rearing process, the parasitized larva may die before it reaches the mature stages. In addition,morphological identification of the parasitoid will be difficult or impossible because lack of an identification key (Walton *et al*., 1990). Besides this, traditional methods are labour intensive and time consuming (Tilmon *et al*., 2000). Molecular identification is very important for precise and accurate results of cryptic species and immature samples, especially when the DNA barcode is used as a tool for species identification (Hebert *et al*., 2003). Furthermore, molecular methods can identify insect species at any life cycle stage (Yadong *et al*., 2010).

Polymerase chain reaction (PCR) techniques offer the best alternative to detecting and identifying both pest-parasitoid species. Therefore, it is necessary to practice new methodologies and approaches in the study of host-parasitoid interactions in ecological or biogeographical research (Santos, 2011). New DNA-based methods and DNA barcoding are valuable tools for identifying species at different stages. DNA barcoding is a molecular technique that is very valuable for rapid and precise identification of species interactions based on standardized short-sequence fragments (Jurado-Rivera 2009), specifically in the identification of small, morphologically uniform, cryptic species and their biological remnants (Greenstone, 2006; Smith *et al*., 2006).

MATERIALS AND METHODS

Sample Collection and Dissection

Potential parasitized pupae and larvae of the moth species were collected using random observation by the naked eye in the Fernarium of Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia. All the samples were brought to the laboratory for molecular work (Fig.1a; Fig.1b). The pupae and larvae of the lepidopteran species were dissected to check for parasitism by endoparasitoids. The samples of endoparasitoids from the parasitized pupae or larvae were collected and stored in 90% alcohol for molecular analysis.

DNA Extraction, PCR Amplification and Sequencing Analysis

The DNA of the pest and parasitoid were extracted using the general protocols provided by QIAGEN DNeasy Blood and Tissue Kit. Meanwhile, universal primers HCO1490 and LCO2198 (Folmer *et al*. 1994) were used to amplify 715bp of *Cytochrome oxidase subunit I* (*COI*) region for the two species (Fig.2). A polymerase chain reaction (PCR) was performed using a 25 µL reaction mixture consisting of 2.5 µL PCR buffer 10X, 1.3 µL 50 mM $MgCl_2$, 0.5 µL 10 mM dNTPs, 0.5 µL each of 10 pmol/µL primers, 0.5 U *Taq Polymerase* (PROMEGA) and 4 µL of DNA samples (6 ng/uL). The temperature profile for PCR amplification used included an initial denaturation step of 94°C for 3 min, followed by 40 cycles of 60 s at 94°C, 60 s at 47°C, 60 s at 72°C, and a 10 min final extension at 72°C. The PCR products were purified using the Geneaid Gel/PCR DNA Fragments Extraction Kit and followed by sending the purified PCR product to the sequencing service company, First Base Sdn. Bhd. in Selangor, Malaysia, for sequencing analysis.

The sequences obtained from the sequencing company were edited using BioEdit (Hall, 1999) and aligned in ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and manually checked by the naked eyes. The alignment of COI sequences was translated to amino acids using the computer program MEGA 4.0 (Tamura *et al*. 2007). The identification of the sequences was then done by comparing them to a reference library using megaBLAST search in GenBank to get highly similar sequences. The NCBI Database measured the values for maximum score, total score, query covery, E-value, and maximum identical (Altschul *et al*., 1997). Lastly, GenBank sequence submissions were made using the Sequin version 12.30 programme (Benson *et al*., 2012).

RESULTS AND DISCUSSION

The species status of pest and parasitoids was confirmed using molecular techniques. Both pest and parasitoids were extracted to get their DNA in order to determine both species. It is therefore necessary to apply new approaches to identifying the species

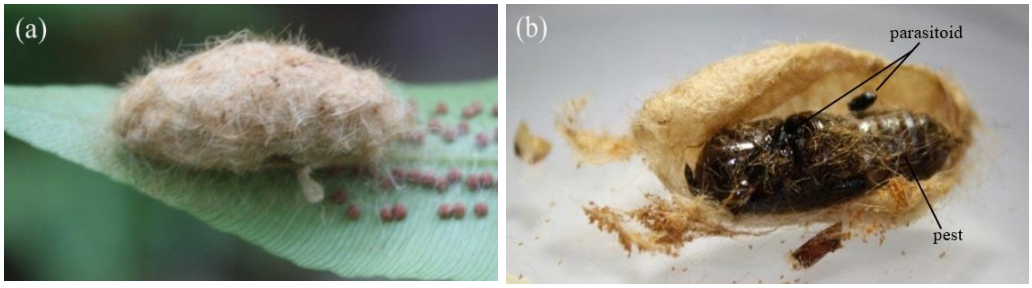


Fig.1(a): Parasitized beet armyworm, *Spodoptera exigua* was found associated with fern, *Shaeropteris mollucana;* (b) The pupae of *Pediobius* sp. were found within the parasitized larva of a beet armyworm.

accurately and rapidly. New DNA-concepts such as DNA barcoding could help the study of host-parasitoid association.

Results of BLAST analysis showed that the *COI* data (KC991186) of the pest was identified as *Spodoptera exigua* with values for maximum score, total score, query covery, E-value, and maximum identical being 941, 941, 95%, 0, and 91%, respectively. For the parasitoid species (KC991185), the results of BLAST showed that the COI referred closely related to *Pediobius* sp. with maximum score, total score, query covery, E-value, and maximum

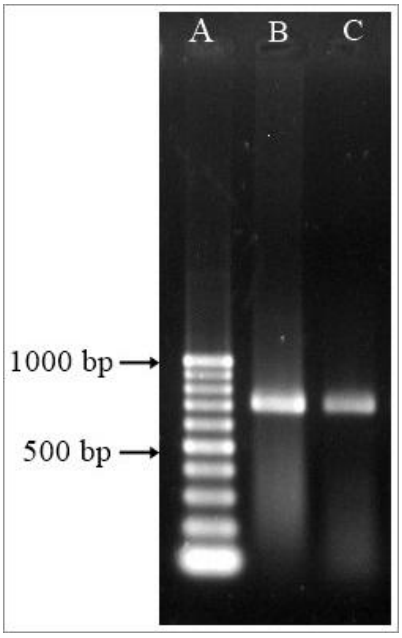


Fig.2: Agarose gel electrophoresis result of PCR product (amplicon size of 715 bp), stained with GelRed and photographed under UV light. Lane A denotes 1 kb ladder; Lane B, PCR product of *Spodoptera exigua*; Lane C, PCR product of *Pediobius* sp.

Fig.3: Immature stages of *Pediobius* sp.

identical being 702, 702, 94%, 0, and 86%, respectively. The low value of E-value for *Pediobius* sp. on the GenBank was because there was no sequence for similar species deposited at the time. Incomplete body development and the immature stages of *Pediobius* sp. meant that further morphological identification at species level was not possible (Fig.3).

This host-parasitoid association has already been previously reported in Iran; *S. exigua* was parasitized by a similar family of hymenopteran insects*, Euplectrus flavipes* (Hymenoptera: Eulophidae) (Talebi *et al*., 2011). In this study, *Pediobius* sp., was found to attack *S. exigua* from a similar family, Eulophidae and this is a new record for Malaysia. The parasitism of this parasitoid species suggests that *Pediobius* sp. could be an important mortality factor in the control of the *S. exigua* population. However, the level of parasitism needs to be taken into account in order to ratify the effectiveness of the integrated pest management programme (IPM). Generally, a detailed IPM is an economical and effective control strategy that minimizes anthropogenic pests using natural components of the agro-ecosystem. An effective strategy of biological control is an important IPM approach to controlling pest outbreaks (Liu *et al*., 2009). A detailed study that includes feeding behaviour, productive behaviour and host specificity is required in order to ensure the effectiveness of a biological control agent.

CONCLUSION

In conclusion, this study indicates that more species databases are needed in GenBank especially in the Barcode of Life Data (BOLD) systems to ensure that molecular identification is easy and effective. Molecular identification was very helpful in this study. Although this interaction was not recorded in the agricultural area, this pest-parasitoid interaction may be able to contribute to future IPM research.

ACKNOWLEDGEMENTS

A special thank goes to Associate Prof. Dr Noraini Talib and Mr Mohamad Ruzi Abd. Rahman for their permission to conduct sampling at the sampling site.This project was fully supported by GGPM-2012-021, ERGS grant/1/2011/STWN/UKM/03/9, UKM-ST-08-FRGS0243-2010 and PTS-2011-032 research grant.

REFERENCES

Aarvik, L. (1981). The migrant moth *Spodoptera exigua* (Hubner) (Lepidoptera, Noctuidae) recorded in Norway. *Fauna Norvegica*, *28,* 90-92.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research, 25*, 3389-3402.

Azidah, A. A. (2007) Population study of *Spodoptera exigua* (Lepidoptera: Noctuidea) Larva and its Affecting Factors in Sekinchan, Selangor. *Pakistan Journal of Biological Sciences, 10*(12), 2152-2158.

Benson, D. A., Karsch-Mizrachi, I., Clark, K., Lipman, D. J., Ostell, J., & Sayer E. W. (2012) GenBank. *Nucleic Acids Research, 40,* D48-D53.

Day, W. H. (1994). Estimating mortality caused by parasites and diseases of insects: comparisons of the dissection and rearing methods. *Entomology Entomology, 23*, 543-550.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial *cytochrome coxidase subunit I* from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294-299.

Greenstone, M. H. (2006) Molecular methods for assessing insect parasitism. *Bulletin of Entomological Research, 96*, 1-13.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hassanein, M. H., Khalil, F. M., & El-Naby, A. A. (1972) Abundance and population density of three lepidopterous insects in the Upper-Egypt (Lepidoptera: Noctuidae). *Bulletin de la Societe Entomologique d'Egypte, 55*, 79-83.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & De Waard J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Soci ety of London Series B: Biological Sciences, 270*, 313–321.

Jurado-Rivera J. A., Vogler, A. P., Reid C. A. M., Petitpierre, E., & Go´mez-Zurita, J. (2009) DNA barcoding insect-host plant associations. Philosophical Transactions of the Royal Society, Series B*, Biological Sciences, 276,* 639-648.

Liu T. X., Kang L., Heinz K. M., & Trumble J. (2009) Biological control of Liriomyza leafminers: Progress and perspective. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4*(4), doi: 10.1079/PAVSNNR20094004.

Pearson, A. C. (1982). *Biology, population dynamics, and pest status of the beet armyworm (Spodoptera exigua) in the Imperial Valley of California*. Ph.D. dissertation, Univ. of California, Riverside Calif.

Ruberson, J., Gary, R., Herzog, A., Lambert, W. R., & Lewis, J. W. (1994). Management of the beet armyworm (Lepidoptera: Noctuidea) in cotton: role of natural enemies. *Florida Entomologist, 77*(4), 440-453.

Santos, A. M. C., Besnard, G., & Quicke, D. L. J. (2011). Applying DNA barcoding for the study of geographical variation in host-parasitoid interactions. *Molecular Ecology Resources, 11*(1), 46-59.

Sivapragasam, A., & Syed, A. R. (2001). The genus Spodoptera with emphasis on the ecology and natural enemies of the beet armyworm, *Spodoptera exigua* Hubner in Malaysia. *Malaysian Plant Protection Society Newsletter*, pp. 6-7.

Sivapragasam, A., Othman, A. B., Palasubramaniam, K., & Gumbek, M. (2001). Terminal report of PF project on *Spodoptera* with emphasis on *S. exigua* in Malaysia. Workshop on *Spodoptera* in Southeast Asia. 14-16 March 2001. p. 8.

Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences, USA* 103: 3657-3662.

Stewart, S. D., Layton, M. B. Jr., & Williams, M. R. (1996). Occurrence and control of beet armyworm outbreaks in the cotton belt In P. Dugger and D. Richter (Eds.), *Proceedings, Beltwide Cotton Conference* (pp. 846-848). National Cotton Council of America, Memphis, TN.

Talebi, A. A., Khoramabadi, A. M., & Rakhshani, E. (2011) Checklist of eulophid wasps (Insecta : Hymenoptera : Eulophidae) of Iran. *Journal of Species Lists and Distribution,* 708-719.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*, 1596-1599.

Tilmon, K. J., Danforth, B. N., Day, W. H., & Hoffmann, M. P. (2000) Determining parasitoid species composition in a host population: a molecular approach. *Annals of the Entomological Society of America, 93*, 640-647.

Tisdale, R. A., & Sappington, T. W. (2001) Realized and potential fecundity, egg fertility, and longevity of laboratory-reared female beet armyworm (Lepidoptera: Noctuidae) under different adult diet regimes. *Annals of the Entomological Society of America, 94*(3), 415-419.

Walton, M. P., Powell, W., Loxdale, H. D., & Allen-Williams, L. (1990). Electrophoresis as a tool for estimating levels of hymenopterous parasitism in Weld populations of the cereal aphid, *Sitobion avenae*. *Entomologia Experimentalis Et Applicata, 54*, 271-279.

Wilson, J. W. (1932). Notes on the biology of *Laphygma exigua* Hübner. *The Florida Entomologist, 16*, 33-39.

Yadong, G., Jifeng, C., Xinghua, W., Lingmei, L., Qinlai, L., Xiang, L., Yunfeng, L., Zhong, M., Xiang, W., & Jifang, W. (2010). Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) based on COI gene in China. *Romanian Society of Legal Medicine, 18*, 217-224.

REFEREES FOR THE PERTANIKA
JOURNAL OF TROPICAL AGRICULTURAL SCIENCE

VOL. 37(2) MAY 2014

The Editorial Board of the Journal of Tropical Agricultural Science wishes to thank the following:

Abdul Rahman Omar
(UPM, Malaysia)

Anuar Abd Rahim
(UPM, Malaysia)

Choong Chee Yen
(UKM, Malaysia)

Chuah Tse Seng
(UMT, Malaysia)

Dahlan Ismail
(UPM, Malaysia)

Darah Ibrahim
(USM, Malaysia)

Edmund Sim Ui Hang
(UNIMAS, Malaysia)

Hajime Ohno
(Shizuoka University, Japan)

Hollena Nori
(UNIMAS, Malaysia)

Ismail Sahid
(UKM, Malaysia)

L Rajendran
(Horticultural Research Station, India)

M Monjurul Alam Mondal
(UPM, Malaysia)

Mohamed Ariff Omar
(UPM, Malaysia)

Mohd Affendy Abd Wahid
(UMT, Malaysia)

Mohd Ridzwan Abd Halim
(UPM, Malaysia)

Prasad K Bhaskaran
(Indian Institute of Technology, Kharagpur, India)

Rayadurga Anantha Sreepada
(National Institute of Oceanography, India)

Tan Soon Guan
(UPM, Malaysia)

Yeap Swee Keong
(UPM, Malaysia)

Z A Ansari
(National Institute of Oceanography, India)

Zaharah A Rahman
(UPM, Malaysia)

Zora Singh
(Curtin University, Australia)

UPM- Universiti Putra Malaysia
UKM- Universiti Kebangsaan Malaysia
USM- Universiti Sains Malaysia
UMT- Universiti Malaysia Terengganu
UNIMAS- Universiti Malaysia Sarawak

While every effort has been made to include a complete list of referees for the period stated above, however if any name(s) have been omitted unintentionally or spelt incorrectly, please notify the Chief Executive Editor, UPM Journals at nayan@upm.my.

Any inclusion or exclusion of name(s) on this page does not commit the *Pertanika* Editorial Office, nor the UPM Press or the University to provide any liability for whatsoever reason.

Pertanika

Our goal is to bring high quality research to the widest possible audience

Journal of Social Sciences and Humanities

INSTRUCTIONS TO AUTHORS

(Manuscript Preparation & Submission Guidelines)

Revised: February 2013

We aim for excellence, sustained by a responsible and professional approach to journal publishing.
We value and support our authors in the research community.

Please read the guidelines and follow these instructions carefully; doing so will ensure that the publication of your manuscript is as rapid and efficient as possible. The Editorial Board reserves the right to return manuscripts that are not prepared in accordance with these guidelines.

About the Journal

Pertanika is an international peer-reviewed journal devoted to the publication of original papers, and it serves as a forum for practical approaches to improving quality in issues pertaining to tropical agriculture and its related fields. *Pertanika* began publication in 1978 as Journal of Tropical Agricultural Science. In 1992, a decision was made to streamline *Pertanika* into three journals to meet the need for specialised journals in areas of study aligned with the interdisciplinary strengths of the university. The revamped Journal of Social Sciences & Humanities (JSSH) aims to develop as a pioneer journal for the Social Sciences with a focus on emerging issues pertaining to the social and behavioural sciences as well as the humanities, particularly in the Asia Pacific region. Other *Pertanika* series include Journal of Tropical Agricultural Science (JTAS); and Journal of Science and Technology (JST).

JSSH is published in **English** and it is open to authors around the world regardless of the nationality. It is currently published four times a year i.e. in **March**, **June**, **September** and **December**.

Goal of *Pertanika*

Our goal is to bring the highest quality research to the widest possible audience.

Quality

We aim for excellence, sustained by a responsible and professional approach to journal publishing. Submissions are guaranteed to receive a decision within 12 weeks. The elapsed time from submission to publication for the articles averages 5-6 months.

Indexing of *Pertanika*

Pertanika is now over 33 years old; this accumulated knowledge has resulted Pertanika JSSH being indexed in SCOPUS (Elsevier), EBSCO, Thomson (ISI) Web of Knowledge [CAB Abstracts], DOAJ, Google Scholar, ISC, Citefactor, Rubriq and MyAIS.

Future vision

We are continuously improving access to our journal archives, content, and research services. We have the drive to realise exciting new horizons that will benefit not only the academic community, but society itself.

We also have views on the future of our journals. The emergence of the online medium as the predominant vehicle for the 'consumption' and distribution of much academic research will be the ultimate instrument in the dissemination of research news to our scientists and readers.

Aims and scope

Pertanika Journal of Social Sciences & Humanities aims to develop as a pioneer journal for the social sciences with a focus on emerging issues pertaining to the social and behavioural sciences as well as the humanities. Areas relevant to the scope of the journal include Social Sciences—Accounting, anthropology, Archaeology and history, Architecture and habitat, Consumer and family economics, Economics, Education, Finance, Geography, Law, Management studies, Media and communication studies, Political sciences and public policy, Population studies, Psychology, Sociology, Technology management, Tourism; Humanities—Arts and culture, Dance, Historical and civilisation studies, Language and Linguistics, Literature, Music, Philosophy, Religious studies, Sports.

Editorial Statement

Pertanika is the official journal of Universiti Putra Malaysia. The abbreviation for *Pertanika* Journal of Social Sciences & Humanities is *Pertanika J. Soc. Sci. Hum.*

Guidelines for Authors

Publication policies

Pertanika policy prohibits an author from submitting the same manuscript for concurrent consideration by two or more publications. It prohibits as well publication of any manuscript that has already been published either in whole or substantial part elsewhere. It also does not permit publication of manuscript that has been published in full in Proceedings. Please refer to Pertanika's **Code of Ethics** for full details.

Editorial process

Authors are notified on receipt of a manuscript and upon the editorial decision regarding publication.

Manuscript review: Manuscripts deemed suitable for publication are sent to the Editorial Board members and/or other reviewers. We encourage authors to suggest the names of possible reviewers. Notification of the editorial decision is usually provided within to eight to ten weeks from the receipt of manuscript. Publication of solicited manuscripts is not guaranteed. In most cases, manuscripts are accepted conditionally, pending an author's revision of the material.

Author approval: Authors are responsible for all statements in articles, including changes made by editors. The liaison author must be available for consultation with an editor of *The Journal* to answer questions during the editorial process and to approve the edited copy. Authors receive edited typescript (not galley proofs) for final approval. Changes **cannot** be made to the copy after the edited version has been approved.

Manuscript preparation

Pertanika accepts submission of mainly four types of manuscripts. Each manuscript is classified as **regular** or **original** articles, **short communications**, **reviews**, and proposals for **special issues**. Articles must be in **English** and they must be competently written and argued in clear and concise grammatical English. Acceptable English usage and syntax are expected. Do not use slang, jargon, or obscure abbreviations or phrasing. Metric measurement is preferred; equivalent English measurement may be included in parentheses. Always provide the complete form of an acronym/abbreviation the first time it is presented in the text. Contributors are strongly recommended to have the manuscript checked by a colleague with ample experience in writing English manuscripts or an English language editor.

Linguistically hopeless manuscripts will be rejected straightaway (e.g., when the language is so poor that one cannot be sure of what the authors really mean). This process, taken by authors before submission, will greatly facilitate reviewing, and thus publication if the content is acceptable.

The instructions for authors must be followed. Manuscripts not adhering to the instructions will be returned for revision without review. Authors should prepare manuscripts according to the guidelines of *Pertanika*.

1. Regular article

Definition: Full-length original empirical investigations, consisting of introduction, materials and methods, results and discussion, conclusions. Original work must provide references and an explanation on research findings that contain new and significant findings.

Size: Should not exceed 5000 words or 8-10 printed pages (excluding the abstract, references, tables and/or figures). One printed page is roughly equivalent to 3 type-written pages.

2. Short communications

Definition: Significant new information to readers of the Journal in a short but complete form. It is suitable for the publication of technical advance, bioinformatics or insightful findings of plant and animal development and function.

Size: Should not exceed 2000 words or 4 printed pages, is intended for rapid publication. They are not intended for publishing preliminary results or to be a reduced version of Regular Papers or Rapid Papers.

3. Review article

Definition: Critical evaluation of materials about current research that had already been published by organizing, integrating, and evaluating previously published materials. Re-analyses as meta-analysis and systemic reviews are encouraged. Review articles should aim to provide systemic overviews, evaluations and interpretations of research in a given field.

Size: Should not exceed 4000 words or 7-8 printed pages.

4. Special issues

Definition: Usually papers from research presented at a conference, seminar, congress or a symposium.

Size: Should not exceed 5000 words or 8-10 printed pages.

5. Others

Definition: Brief reports, case studies, comments, Letters to the Editor, and replies on previously published articles may be considered.

Size: Should not exceed 2000 words or up to 4 printed pages.

With few exceptions, original manuscripts should not exceed the recommended length of 6 printed pages (about 18 typed pages, double-spaced and in 12-point font, tables and figures included). Printing is expensive, and, for the Journal, postage doubles when an issue exceeds 80 pages. You can understand then that there is little room for flexibility.

Long articles reduce the Journal's possibility to accept other high-quality contributions because of its 80-page restriction. We would like to publish as many good studies as possible, not only a few lengthy ones. (And, who reads overly long articles anyway?) Therefore, in our competition, short and concise manuscripts have a definite advantage.

Format

The paper should be formatted in one column format with at least 4cm margins and 1.5 line spacing throughout. Authors are advised to use Times New Roman 12-point font. Be especially careful when you are inserting special characters, as those inserted in different fonts may be replaced by different characters when converted to PDF files. It is well known that 'μ' will be replaced by other characters when fonts such as 'Symbol' or 'Mincho' are used.

A maximum of eight keywords should be indicated below the abstract to describe the contents of the manuscript. Leave a blank line between each paragraph and between each entry in the list of bibliographic references. Tables should preferably be placed in the same electronic file as the text. Authors should consult a recent issue of the Journal for table layout.

Every page of the manuscript, including the title page, references, tables, etc. should be numbered. However, no reference should be made to page numbers in the text; if necessary, one may refer to sections. Underline words that should be in italics, and do not underline any other words.

We recommend that authors prepare the text as a **Microsoft Word** file.

1. Manuscripts in general should be organised in the following order:

    - **Page 1: Running title.** (Not to exceed 60 characters, counting letters and spaces). This page should **only** contain the running title of your paper. The running title is an abbreviated title used as the running head on every page of the manuscript.

      In addition, the **Subject areas m**ost relevant to the study must be indicated on this page. Select the appropriate subject areas from the Scope of the Journals provided in the Manuscript Submission Guide.

    - A **list** of n**umber of black and white / colour figures and tables** should also be indicated on this page. Figures submitted in color will be printed in colour. See "*5. Figures & Photographs"* for details.

    - **Page 2: Author(s) and Corresponding author information**. This page should contain the **full title** of your paper with name(s) of all the authors, institutions and corresponding author's name, institution and full address (Street address, telephone number (including extension), hand phone number, fax number and e-mail address) for editorial correspondence. The names of the authors **must** be abbreviated following the international naming convention. e.g. Salleh, A.B., Tan, S.G., or Sapuan, S.M.

      Authors' addresses. Multiple authors with different addresses must indicate their respective addresses separately by superscript numbers:

      George Swan[1] and Nayan Kanwal[2]
      [1]Department of Biology, Faculty of Science, Duke University, Durham, North Carolina, USA.
      [2]Office of the Deputy Vice Chancellor (R&I), Universiti Putra Malaysia, Serdang, Malaysia.

    - **Page 3**: This page should **repeat** the **full title** of your paper with only the **Abstract** (the abstract should be less than 250 words for a Regular Paper and up to 100 words for a Short Communication). **Keywords** must also be provided on this page (Not more than eight keywords in alphabetical order).

    - **Page 4 and subsequent pages**: This page should begin with the **Introduction** of your article and the rest of your paper should follow from page 5 onwards.

Abbreviations. Define alphabetically, other than abbreviations that can be used without definition. Words or phrases that are abbreviated in the introduction and following text should be written out in full the first time that they appear in the text, with each abbreviated form in parenthesis. Include the common name or scientific name, or both, of animal and plant materials.

Footnotes. Current addresses of authors if different from heading.

2. **Text.** Regular Papers should be prepared with the headings **Introduction**, **Materials and Methods**, **Results and Discussion, Conclusions** in this order. Short Communications should be prepared according to "*8. Short Communications."* below.

3. **Tables.** All tables should be prepared in a form consistent with recent issues of *Pertanika* and should be numbered consecutively with Arabic numerals. Explanatory material should be given in the table legends and footnotes. Each table should be prepared on a separate page. (Note that when a manuscript is accepted for publication, tables must be submitted as data - .doc, .rtf, Excel or PowerPoint file- because tables submitted as image data cannot be edited for publication.)

4. **Equations and Formulae.** These must be set up clearly and should be typed triple spaced. Numbers identifying equations should be in square brackets and placed on the right margin of the text.

5. **Figures & Photographs.** Submit an original figure or photograph. Line drawings must be clear, with high black and white contrast. Each figure or photograph should be prepared on a separate sheet and numbered consecutively with Arabic numerals. Appropriate sized numbers, letters and symbols should be used, no smaller than 2 mm in size after reduction to single column width (85 mm), 1.5-column width (120 mm) or full 2-column width (175 mm). Failure to comply with these specifications will require new figures and delay in publication. For electronic figures, create your figures using applications that are capable of preparing high resolution TIFF files acceptable for publication. In general, we require **300 dpi or higher resolution for coloured and half-tone artwork** and **1200 dpi or higher for line drawings**.

   For review, you may attach low-resolution figures, which are still clear enough for reviewing, to keep the file of the manuscript under 5 MB. Illustrations may be produced at extra cost in colour at the discretion of the Publisher; the author could be charged Malaysian Ringgit 50 for each colour page.

6. **References.** Literature citations in the text should be made by name(s) of author(s) and year. For references with more than two authors, the name of the first author followed by *'et al*.' should be used.

   Swan and Kanwal (2007) reported that …
   The results have been interpreted (Kanwal *et al.,* 2009).

   - References should be listed in alphabetical order, by the authors' last names. For the same author, or for the same set of authors, references should be arranged chronologically. If there is more than one publication in the same year for the same author(s), the letters 'a', 'b', etc., should be added to the year.

   - When the authors are more than 11, list 5 authors and then et al.

   - Do not use indentations in typing References. Use one line of space to separate each reference. The name of the journal should be written in full. For example:

     - Mellers, B. A. (2006a). Choice and the relative pleasure of consequences. *Psychological Bulletin, 126,* 910-924.
     - Mellers, B. A. (2006b). Treatment for sexually abused children and adolescents. *American Psychologist, 55,* 1040-1049.
     - Hawe, P. (2005). Capturing the meaning of "community" in community intervention evaluation: Some contributions from community psychology. *Health Promotion International, 9,*199-210.
     - Braconier, H., & Ekholm, K. (2006). Swedish multinationals and competition from high and low wage location. *Review of International Economics, 8,* 448-461.

   - In case of citing an author(s) who has published more than one paper in the same year, the papers should be distinguished by addition of a small letter as shown above, e.g. Jalaludin (1997a); Jalaludin (1997b).

   - Unpublished data and personal communications should not be cited as literature citations, but given in the text in parentheses. 'In press' articles that have been accepted for publication may be cited in References. Include in the citation the journal in which the 'in press' article will appear and the publication date, if a date is available.

7. **Examples of other reference citations**:

   Monographs: Kalimapour, Y.R. (2004). *Images of the U.S. Around the World: A Multicultural Perspective*. Albany, NY: State University of New York Press.

   Chapter in Book: Bjork, R. A. (2007). Retrieval inhibition as an adaptive mechanism in human memory. In H. L. Roediger III & F. I. M. Craik (Eds.), *Varieties of memory & consciousness* (pp. 309-330). Hull: Hull University Press.

   - ***Proceedings***: Amir Awang. (2006). Counseling, human resources development and counseling services. In Sulaiman M. Yassin, Yahya Mat Hassan, Kamariah Abu Bakar, Esah Munji and Sabariah Mohd. Rashid (Eds.), *Proceedings of Asia Pacific Conference on Human Development* (p. 243-246). Serdang: Universiti Putra Malaysia.

8. **Short Communications** should include **Introduction, Materials and Methods, Results and Discussion, Conclusions** in this order. Headings should only be inserted for Materials and Methods. The abstract should be up to 100 words, as stated above. Short Communications must be 5 printed pages or less, including all references, figures and tables. References should be less than 30. A 5 page paper is usually approximately 3000 words plus four figures or tables (if each figure or table is less than 1/4 page).

   *Authors should state the total number of words (including the Abstract) in the cover letter. Manuscripts that do not fulfill these criteria will be rejected as Short Communications without review.

STYLE OF THE MANUSCRIPT

Manuscripts should follow the style of the latest version of the Publication Manual of the American Psychological Association (APA). The journal uses American or British spelling and authors may follow the latest edition of the Oxford Advanced Learner's Dictionary for British spellings.

SUBMISSION OF MANUSCRIPTS

All articles should be submitted electronically using the ScholarOne web-based system. ScholarOne, a Thomson Reuters product provides comprehensive workflow management systems for scholarly journals. For more information, go to our web page and click "**Online Submission**".

Alternatively, you may submit the electronic files (cover letter, manuscript, and the **Manuscript Submission Kit** comprising *Declaration* and *Referral* form) via email directly to the Executive Editor. If the files are too large to email, mail a CD containing the files. The **Manuscript Submission Guide** and **Submission Kit** are available from the *Pertanika*'s home page at http://www.pertanika.upm.edu.my/home.php or from the Chief Executive Editor's office upon request.

All articles submitted to the journal **must comply** with these instructions. Failure to do so will result in return of the manuscript and possible delay in publication.

Please do **not** submit manuscripts to the editor-in-chief or to any other office directly. All manuscripts must be **submitted through the chief executive editor's office** to be properly acknowledged and rapidly processed at the address below:

Dr. Nayan KANWAL
Chief Executive Editor
Pertanika Journals, UPM Press
Office of the Deputy Vice Chancellor (R&I)
IDEA Tower II, UPM-MTDC Technology Centre
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor,
Malaysia

E-mail: nayan@upm.my; journal.officer@gmail.com tel: + 603-8947 1622
or visit our website at http://www.pertanika.upm.edu.my/ for further information.

Authors should retain copies of submitted manuscripts and correspondence, as materials can not be returned. Authors are required to inform the Chief Executive Editor of any change of address which occurs whilst their papers are in the process of publication.

Cover letter

All submissions must be accompanied by a cover letter detailing what you are submitting. Papers are accepted for publication in the journal on the understanding that the article is original and the content has not been published or submitted for publication elsewhere. This must be stated in the cover letter.

The cover letter must also contain an acknowledgement that all authors have contributed significantly, and that all authors are in agreement with the content of the manuscript.

The cover letter of the paper should contain (i) the title; (ii) the full names of the authors; (iii) the addresses of the institutions at which the work was carried out together with (iv) the full postal and email address, plus facsimile and telephone numbers of the author to whom correspondence about the manuscript should be sent. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

As articles are double-blind reviewed, material that might identify authorship of the paper should be placed on a cover sheet.

Peer review

Pertanika follows a **double-blind peer-review** process. Peer reviewers are experts chosen by journal editors to provide written assessment of the **strengths** and **weaknesses** of written research, with the aim of improving the reporting of research and identifying the most appropriate and highest quality material for the journal.

In the peer-review process, three referees independently evaluate the scientific quality of the submitted manuscripts. Authors are encouraged to indicate in the **Referral form** using the **Manuscript Submission Kit** the names of three potential reviewers, but the editors will make the final choice. The editors are not, however, bound by these suggestions..

Manuscripts should be written so that they are intelligible to the professional reader who is not a specialist in the particular field. They should be written in a clear, concise, direct style. Where contributions are judged as acceptable for publication on the basis of content, the Editor reserves the right to modify the typescripts to eliminate ambiguity and repetition, and improve communication between author and reader. If extensive alterations are required, the manuscript will be returned to the author for revision.

The Journal's review process

What happens to a manuscript once it is submitted to *Pertanika*? Typically, there are seven steps to the editorial review process:

1. The executive editor and the editorial board examine the paper to determine whether it is appropriate for the journal and should be reviewed. If not appropriate, the manuscript is rejected outright and the author is informed.

2. The executive editor sends the article-identifying information having been removed, to three reviewers. Typically, one of these is from the Journal's editorial board. Others are specialists in the subject matter represented by the article. The executive editor asks them to complete the review in three weeks and encloses two forms: (a) referral form B and (b) reviewer's comment form along with reviewer's guidelines. Comments to authors are about the appropriateness and adequacy of the theoretical or conceptual framework, literature review, method, results and discussion, and conclusions. Reviewers often include suggestions for strengthening of the manuscript. Comments to the editor are in the nature of the significance of the work and its potential contribution to the literature.

3. The executive editor, in consultation with the editor-in-chief, examines the reviews and decides whether to reject the manuscript, invite the author(s) to revise and resubmit the manuscript, or seek additional reviews. Final acceptance or rejection rests with the Editorial Board, who reserves the right to refuse any material for publication. In rare instances, the manuscript is accepted with almost no revision. Almost without exception, reviewers' comments (to the author) are forwarded to the author. If a revision is indicated, the editor provides guidelines for attending to the reviewers' suggestions and perhaps additional advice about revising the manuscript.

4. The authors decide whether and how to address the reviewers' comments and criticisms and the editor's concerns. The authors submit a revised version of the paper to the executive editor along with specific information describing how they have answered' the concerns of the reviewers and the editor.

5. The executive editor sends the revised paper out for review. Typically, at least one of the original reviewers will be asked to examine the article.

6. When the reviewers have completed their work, the executive editor in consultation with the editorial board and the editor-in-chief examine their comments and decide whether the paper is ready to be published, needs another round of revisions, or should be rejected.

7. If the decision is to accept, the paper is sent to that Press and the article should appear in print in approximately three months. The Publisher ensures that the paper adheres to the correct style (in-text citations, the reference list, and tables are typical areas of concern, clarity, and grammar). The authors are asked to respond to any queries by the Publisher. Following these corrections, page proofs are mailed to the corresponding authors for their final approval. At this point, only essential changes are accepted. Finally, the article appears in the pages of the Journal and is posted on-line.

English language editing

Pertanika **emphasizes** on the linguistic accuracy of every manuscript published. Thus all authors are required to get their manuscripts edited by **professional English language editors**. Author(s) **must provide a certificate** confirming that their manuscripts have been adequately edited. A proof from a recognised editing service should be submitted together with the cover letter at the time of submitting a manuscript to Pertanika. **All costs will be borne by the author(s)**.

This step, taken by authors before submission, will greatly facilitate reviewing, and thus publication if the content is acceptable.

Author material archive policy

Authors who require the return of any submitted material that is rejected for publication in the journal should indicate on the cover letter. If no indication is given, that author's material should be returned, the Editorial Office will dispose of all hardcopy and electronic material.

Copyright

Authors publishing the Journal will be asked to sign a declaration form. In signing the form, it is assumed that authors have obtained permission to use any copyrighted or previously published material. All authors must read and agree to the conditions outlined in the form, and must sign the form or agree that the corresponding author can sign on their behalf. Articles cannot be published until a signed form has been received.

Lag time

A decision on acceptance or rejection of a manuscript is reached in 3 to 4 months (average 14 weeks). The elapsed time from submission to publication for the articles averages 5-6 months.

Hardcopies of the Journals and off prints

Under the Journal's open access initiative, authors can choose to download free material (via PDF link) from any of the journal issues from Pertanika's website. Under "Browse Journals" you will see a link entitled "Current Issues" or "Archives". Here you will get access to all back-issues from 1978 onwards.

The **corresponding author** for all articles will receive one complimentary hardcopy of the journal in which his/her articles is published. In addition, 20 off prints of the full text of their article will also be provided. Additional copies of the journals may be purchased by writing to the executive editor.

BACKGROUND

Pertanika began publication in 1978 as the Journal of Tropical Agricultural Science (JTAS).

In 1992, a decision was made to streamline *Pertanika* into **3 journals**. i.e.,

1. Journal of Tropical Agricultural Science (JTAS)
2. Journal of Science and Technology (JST)
3. Journal of Social Sciences and Humanities (JSSH)

BENEFITS TO AUTHORS

PROFILE: *Pertanika* publishes original academic articles rapidly. It is fully committed to the Open Access Initiative and provides free access to all articles as soon as they are published.

QUALITY: Articles submitted to *Pertanika* undergo rigid originality checks. Our double-blind peer review procedures are fair and open.

AUTHOR SERVICES: We ensure that your work reaches the widest possible audience in print and online rapidly. Submissions are through **ScholarOne** system by Thomson Reuters.

SUBMISSION GUIDELINES

The Journal accepts articles as **regular**, **short communication** or **review papers**.

The article should include the following:

- An abstract of not more than 300 words;
- Up to 8 related keywords;
- Name(s), Institutional affiliation(s) and email(s) of each author.
- The maximum length of your article must not exceed:
  - approximately 6000 words or 27 pages, including abstract, diagrams tables and references for full research papers,
  - 2000 words for short communication papers, or
  - 4000 words for review papers
- References should be listed in APA style.

SUBMISSION DEADLINE

You may submit your articles at any time of the year. The journal is now accepting papers for its 2013-14 issues.

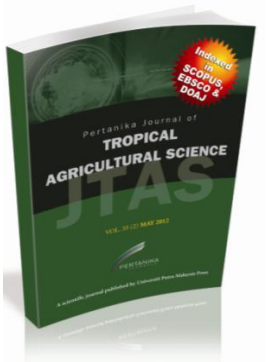
CONTACT US

For guidance on the submission process, or for any questions regarding submissions, you may contact the **Chief Executive Editor** at: nayan@upm.my

Call for Papers 2014-15

now accepting submissions…

About the Journal



- An international multidisciplinary peer-reviewed leading Malaysian journal.
- Publishes articles in **English** quarterly. i.e., *February, May, August* and *November*.
- The elapsed time from submission to publication for the articles averages 5 to 6 months. A decision on acceptance of a manuscript is reached in 3 to 4 months (average 14 weeks).
- Indexed in **SCOPUS** (Elsevier), **Thomson (ISI) Web of Knowledge** [CAB Abstracts], **EBSCO**, **DOAJ**, **Agricola**, **CABI**, **Google Scholar**, **MyAIS**, **ISC** & **Rubriq**.

Scope of Journal

- Pertanika JTAS aims to provide a forum for high-quality research related to **tropical agricultural research** dealing with issues of worldwide relevance.
- Refer to our website for detailed scope areas. http://www.pertanika.upm.edu.my/scope.php

Format for Paper Submission

- Articles should include the following:
  - problem formulation
  - conceptual framework
  - methodology/ approach
  - research design (if applicable)
  - statistical analysis (if applicable)
  - main findings
  - overall contribution
  - conclusions & suggestion for further research
  - acknowledgements (if applicable)

Rapid research publication…

Pertanika is the resource to support you in strengthening your research.

View current issue

View journal archives

Submit your manuscript to
http://mc.manuscriptcentral.com/upm-jtas

PERTANIKA JOURNALS

Journal's profile: http://www.pertanika.upm.edu.my/

Pertanika Journal of Tropical Agricultural Science
Vol. 37 (2) May 2014

Contents

Foreword

Nayan Deep S. Kanwal i

Review Article

Senescence and Postharvest Studies of Cut Flowers: A Critical Review 159
Pooja Rani and Narender Singh

Regular Articles

The Enzyme Activities of Pancreas and Small Intestinal Contents in the Malaysian Village Chicken and Broiler Strains 203
Khalid K. Kadhim, Md Zuki Abu Bakar, Noordin Mohamed Mustapha, Mohd Amin Babjee and Mohd Zamri Saad

The Responses by Gut-Associated and Bronchus-Associated Lymphoid Tissues of Buffalo Calves Following Oral Exposure to *Pasteurella multocida* B:2 215
M. S. Abu Bakar, Mohd Zamri Saad, S. Jasni and Zuki Abu Bakar

Increasing Rice Production Using Different Lime Sources on an Acid Sulphate Soil in Merbok, Malaysia 223
Elisa Azura Azman, Shamshuddin Jusop, Che Fauziah Ishak and Roslan Ismail

Cattle Grazing Effect on *Mimosa pudica* L. in Tropical Pasture System 249
Majid Ajorlo, Ramdzani Abdullah, Ridzwan Abdul Halim and Mahboubeh Ebrahimian

Stored Carbon in Dominant Seaweeds of Indian Sundarbans 263
Mitra, A., Zaman, S., Pramanick, P., Bhattacharyya, S. B. and Raha, A. K.

Mixed Viral Infection and Growth Stage on Chilli (*Capsicum annuum* L.) Production 275
Nurhayati Damiri

Determination of *Pediobius* sp. (Hymenoptera: Eulophidae), A New Species Record of Endoparasitoid Associate with Beet Armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidea) from Malaysia using DNA Barcode 285
Ghazali, S. Z., Md-Zain, B. M. and Yaakop, S.

Pertanika Editorial Office, Journal Division
Office of the Deputy Vice Chancellor (R&I)
1st Floor, IDEA Tower II
UPM-MTDC Technology Centre
Universiti Putra Malaysia
43400 UPM Serdang
Selangor Darul Ehsan
Malaysia

http://www.pertanika.upm.edu.my/
E-mail: executive_editor.pertanika@upm.my
Tel: +603 8947 1622 / 1619

PENERBIT UPM UNIVERSITI PUTRA MALAYSIA PRESS

http://penerbit.upm.edu.my
E-mail : penerbit@putra.upm.edu.my
Tel : +603 8946 8855 / 8854
Fax : +603 8941 6172

ISSN 1511-3701
05 >
9 771511 370234