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Genetic Identification and Micropropagation of Distributed Persimmons (*Diospyros kaki*) in Indonesia

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ABSTRACT

While existing persimmon productivity in Indonesia has been gradually decreasing because of improper cultural practices and environmental conditions, species conservation has become increasingly crucial given its benefit to human health. Genetic identification of the persimmon (*Diospyros kaki*), the type found in Indonesia, would provide valuable genetic information about this persimmon cultivar. Nine selected random amplified polymorphic DNA (RAPD) primers successfully yielded 32 polymorphic bands and 20 monomorphic bands. Amplified bands identified 20 persimmon samples that were derived from one genotype, given their similarity coefficient value of more than 0.76. The genotype was cultured under a controlled environment in order to produce clonal and healthy seedlings over a year. Although persimmon micropropagation had been successfully established at the initiation stage, but the explants growth of each treatment did not significantly differ. However, the highest mean of leaf number was achieved individually on treatment of Woody Plant Medium (WPM) and plant growth regulator (IBA 2 ppm + BAP 2 ppm); this is significant given that the number of formed leaves determines the persimmon multiplication rate. The regenerated shoot must be subcultured

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on modified culture medium for further persimmon micropropagation stages.

Keywords: Conservation, *Diospyros kaki*, genetic identification, micropropagation

INTRODUCTION

The weather and topographical conditions of Indonesia provide a supportive habitat for the growth of diverse germplasms, and one of the valuable species produced in this diversity is the persimmon. According to Ng (as cited in Santosa et al., 2005, p. 220), persimmons are native to China and were introduced to Indonesia about one hundred years ago. Persimmons that are distributed in Indonesia grow in areas that are at least 1,000 m above sea level, such as Selo (Central Java province) and Magetan (East Java province; Figure 1a). There are many genotypes of persimmon, but *Diospyros kaki* is the most widely distributed species for commercial purposes (Yonemori et al., 2000). Of the two types of persimmons, astringent or non-astringent, those found in Indonesia belong to the astringent type, which requires astringency removal treatment prior to consumption (Figure 1b). Compared with the non-astringent type, it can only be consumed directly when ripe. The fruit and leaves of persimmons are great sources of natural antioxidants and have been studied with regard to human health needs (Butt et al., 2015; Yaqub et al., 2016).

The persimmons distributed in Selo (Central Java province) and Magetan (East Java province) are approximately 80 years old, but unfortunately, fruit production is gradually decreasing because of improper cultural practices and environmental conditions. Therefore, conservation must be conducted in order to protect the particular persimmon genotype of Indonesia, and proper cultivation is required to enhance existing persimmon production. Genetic identification is a beginning step toward conservation establishment; to this end, a molecular marker can be used to identify relationships and genetic variations among

Figure 1. (a) Growth habitat; and (b) persimmon fruit appearance

persimmons, as genetic variation is needed to select desired traits for the next generations. Random amplified polymorphic DNA (RAPD) primers comprise one of the most commonly used methods for DNA marker identification in a manner that is simple and rapid as well as cost- and time-effective. RAPD primers bind randomly on the complementary sequence of the DNA target to amplify large amounts of genomic DNA, and presence or absence of amplified bands in individual lanes is used to determine genetic diversity (William et al., 1990). The genetic diversity among the persimmons in Italy, Japan, Korea, China, Spain and Turkey has been successfully investigated earlier using various molecular markers (Badenes et al., 2003; del Mar Naval et al., 2010; Guo & Luo, 2011; Parfitt et al., 2015; Yamagishi et al., 2005; Yonemori et al., 2008). This research presents the first report of distributed persimmon genetic identification in Indonesia.

Moving a step further toward conservation, micropropagation was performed in order to provide high-quality persimmon seedlings for productivity improvement. Micropropagation is a promising tool for clonal seedling production within a limited space that does not harm the donor plant (Bhojwani & Razdan, 1996; Bonga & Von Aderkas, 1992). The method could produce a healthy plant over the course of a year. The lateral meristem was chosen as an explant because of its higher ability for shoot multiplication. Various persimmon varieties have been successfully cultured on different culture media. A half-strength Murashige Skoog (MS) medium supplemented with various plant growth regulators has successfully induced regeneration of *Diospyros kaki* Thunb. cv Hachiya, *Diospyros kaki* L., *Diospyros kaki* Thunb., *Diospyros japonica*, *Diospyros lotus* and *Diospyros virginiana* (Fukui et al., 1992; Kochanova et al., 2011; Tao & Sugiura, 1992). Alternatively, *D. kaki* Thunb. cv. 'Nishimurawase' was successfully established on Woody Plant Medium (WPM) supplemented with zeatin 10^{-5} M (Fukui et al., 1989). Based on the above results, ½ N MS and WPM supplemented with indole-3-butryric acid (IBA) and 6-benzylaminopurine (BAP) were tested to determine the best culture condition for persimmons in Indonesia.

MATERIALS AND METHODS

Genetic Identification by RAPD Marker

Sample Collection. Persimmon (*D. kaki* L.) leaves were collected from:

- Central Java province, which consisted of three villages:
  - Sened, located at 7°29'51.4"S 110°28'32.1"E and 1,461 m above sea level;
  - Sepandan Lor, located at 7°29'59.4"S 110°29'18.18"E and 1,327 m above sea level; and
  - Gebyog, located at 7°29'57.4"S 110°28'16.1"E and 1,499 m above sea level.
- East Java province, which consisted of:
  - Magetan, located at 7°48'45.97"S 111°2'43.3"E and 1,314 m above sea level.

The distance between the three villages in Central Java province is 1–2 km of each other. The distance of East Java province is 137 km from Central Java province. Five persimmon trees were randomly selected from each village. Leaves were taken from persimmon trees in:

- Sened, coded as S1, S2, S3, S4 and S5;
- Sepandan Lor, coded as SL1, SL2, SL3, SL4 and SL5;
- Gebyog coded as G1, G2, G3, G4 and G5; and
- Magetan coded as M1, M2, M3, M4 and M5.

DNA Extraction. Genomic DNA was extracted from frozen mature persimmon leaves via the cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1987). A working DNA concentration of 50 ng/µL was prepared and stored at 4°C until use.

RAPD Analysis. Twenty oligonucleotides (Operon Technologies) were initially screened in order to select primers that yielded clear, reproducible bands and polymorphism detection. Selected primers are listed in Table 1. PCR reactions were performed in a volume of 10-µl mix solution of GoTaq® Green Master Mix (Promega) 5 µl, nuclease-free Water 2 µl, primer 0.5 µl and DNA 2.5 µl. PCR conditions were 95°C for 5 min, 40 cycles at [95°C for 45 s, 37°C for 1.45 s, 72°C for 45 s] and 72°C for 7 min. PCR products were electrophoresed on 1.5% agarose gel containing 4 µl DNA stain and visualized on a UV transilluminator. Amplified bands were scored to construct dendrograms using NTSYS 2.02 software analysed by unweighted pair group method with arithmetic average (UPGMA). The similarity coefficient of dendrograms determined the genetic relationship among the persimmons.

Table 1

The bands reproducibility were generated by RAPD primers

No.	Primer	Sequence (5'-3')	Number of amplified bands	Number of polymorphic bands	Polymorphism (%)
1	OPA 5	AGGGGTCTTG	4	1	25.0
2	OPA 18	AGGTGACCGT	4	2	50.0
3	OPC 11	AAAGCTGCGG	4	2	50.0
4	OPD 2	GGACCCAACC	7	5	71.4
5	OPD 8	GTGTGCCCCA	5	4	80.0
6	OPD 11	AGCGCCATTG	7	5	71.4
7	OPD 13	ACGCGCATGT	6	5	83.3
8	OPD 18	GAGAGCCAAC	8	2	25.0
9	OPD 20	ACCCGGTCAC	7	6	85.7
	Total		52	32	61.5

Micropropagation Protocol

Lateral shoots derived from mature persimmon trees in Sened village were used as the explant because they had the highest regenerated explants rate of *in vitro* establishment (Table 2). The cut lateral shoots were cleaned with alcohol 70% and sprayed with a mix solution of fungicide 'Dithane' (1.5 g/100 ml) and bactericide 'Agrept' (1 g/100 ml). The lateral shoots were delivered from the field to the Laboratorium of Plant Physiology and Biotechnology, Agriculture Faculty, Sebelas Maret University.

At the laboratory, lateral shoots were washed under running tap water for 5 minutes and immersed into bactericide 'Agrept' (1.5 g/100 ml) and fungicide 'Dithane' (1.5 g/100 ml) solutions for 20 minutes; they were then rinsed in distilled water five times and placed in laminar air flow (LAF) for further sterilization with sterile distilled water two times. Surface sterilization of the lateral shoots was carried out aseptically by immersion into NaOCl 4% for 4 minutes (Wardani et al., 2019). Afterwards, lateral shoots were cut into single nodes (2-3 cm) and then immersed into ascorbic acid 10% for 20 minutes. Finally, lateral shoots were rinsed again with sterile distilled water three times. Explants were cultured on half-strength (½) Murashige-Skoog Medium (M1) and Woody Plant Medium (M2) supplemented with:

Z1 : IBA 1 ppm + BAP 4 ppm
Z2 : IBA 2 ppm + BAP 2 ppm
Z3 : IBA 3 ppm + BAP 2 ppm

There were six treatments. Due to high contamination and browning, each treatment only consisted of one sample with three replications. Samples were moved to the culture room and maintained at 24°C with a 24-h photoperiod using white fluorescent lighting. Explant growth consisted of callus and leaf formation noted for two months and analysed using SPSS software. Analysis of variance (ANOVA) value indicated no statistical significance for each treatment, so further analysis with Duncan's was not required.

Table 2

The regeneration rate of each persimmon sample

Sample	Browning (dead explants)	Contamination	Regenerated explants
Sened	29.63%	37.04%	33.33%
Sepandan lor	53.70%	46.30%	0%
Gebyog	20.37%	79.63%	0%
Magetan	44.44%	55.56%	0%

RESULT

Genetic Identification by RAPD

The nine selected primers yielded a total of 52 bands ranging from 125 bp to 1.2 kb, and 32 of them were recorded as polymorphic bands (Table 1). The highest number of amplified bands yielded by OPD 18 (8 bands), but only two polymorphic bands were apparent. Its polymorphism rate – 25% – was the same rate generated by OPA 5, while a polymorphism rate of 50% was generated by OPA 18 and OPC 11. The five RAPD primers (OPD 2, OPD 8, OPD 11, OPD 13 and OPD 20) successfully detected more than 50% of polymorphisms among the persimmons; the highest polymorphic bands could be obtained by OPD 20 (Figure 2).

Two main clusters were formed based on the similarity coefficient value (Figure 3). The first main cluster only contains SL5 with a similarity coefficient value of 0.76 to the second main cluster. Nineteen persimmon samples constructed the second main cluster. Genetic mutations may occur on SL5 but were still closely related to the other samples. The second main cluster consisted of two groups: the first group consisted of fifteen persimmon samples from Central Java, and the second group consisted of five persimmon samples from East Java. The similarity coefficient value for both groups was 0.78, indicating that all of the persimmon samples were derived from the same genotype, even though they were located in different provinces. The first group consisted of many subgroups that had similarity coefficient values of more than 0.86; only S1 and S5 were genetically identical samples. The second group also consisted of many subgroups that had a similarity coefficient value of more than 0.886. Both values indicated a close genetic relationship among the persimmons. The genetic variation among the samples was very low, probably because of all persimmons derived from the same genotype.

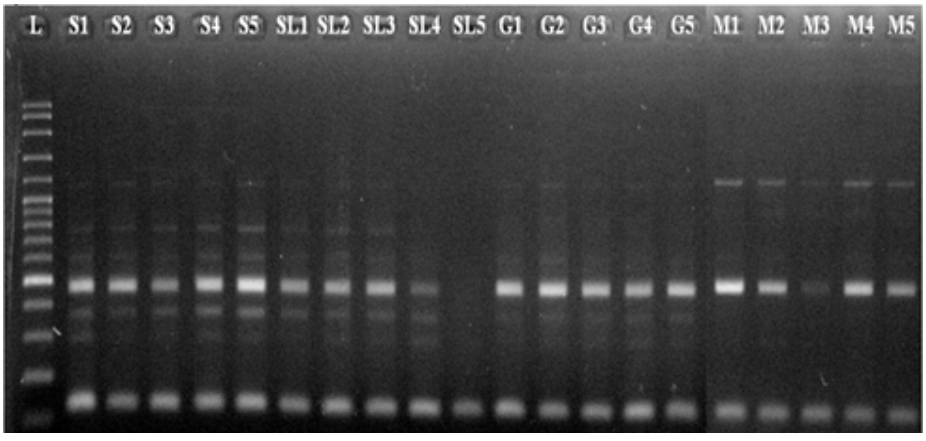


Figure 2. Amplified bands generated by the primer OPD 20. M: 100 bp DNA ladder

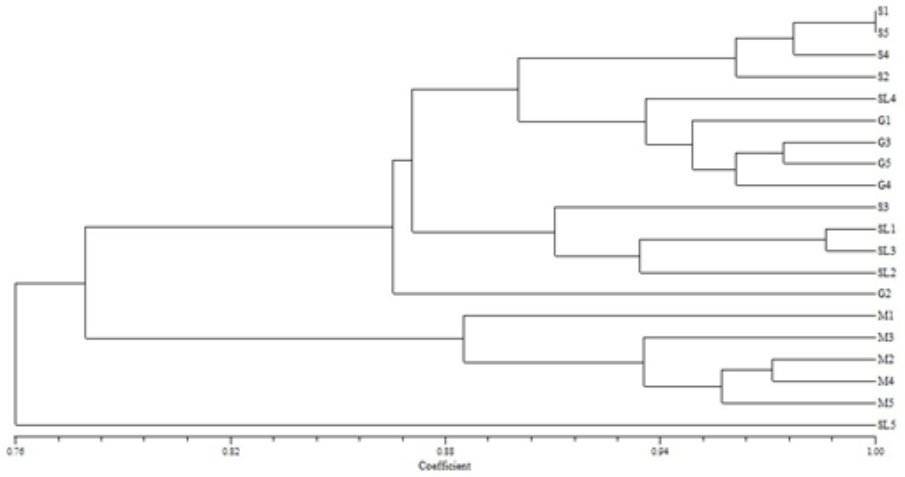


Figure 3. Genetic relationship among the persimmons

Establishment of Persimmon Micropropagation

The micropropagation protocol of persimmon genotype in Indonesia has been established in this study. The lateral shoots of persimmons were successfully cultured on MS (½ N) and WPM supplemented with various combinations of BAP and IBA. Based on ANOVA, there was no interaction between culture medium types containing various plant growth regulators toward explant regeneration (Table 3). In addition, each treatment did not significantly affect explant regeneration. Each explant had an existing bud that formed leaves after more than 50 days of culturing, and leaves induction was obtained without the interference of callus formation (Figure 4a).

A few explants formed callus at the basal stem in response to wounding stress. In addition, all treatments produced non-embryogenic callus. Based on the information in Table 3, the callus emergence of each treatment was not significantly different. The higher percentage of the non-embryogenic callus formation (55%) has no ability to form leaves, resulting in a decrease in the means of leaf number and length of each treatment. However, the highest mean of leaf number (2 leaves) was achieved individually on WPM and plant growth regulator (IBA 2 ppm + BAP 2 ppm), which is noteworthy given that the number of formed leaves determines persimmon multiplication rate.

The highest mean of leaf length was successfully induced on ½ MS of 3.21 mm and plant growth regulator (IBA 2 ppm + BAP 2 ppm) of 3.55 mm. Based on the correlation test, the early emergence of leaves enhanced the number and length of leaves and increasing the number of leaves had a positive correlation with the length of leaves.

After the seventh week of culturing, the white and friable non-embryogenic callus

turned brown and leaves became necrotic (Figure 4b). The culture medium also turned dark brown. This could have resulted from phenolic accumulation causing growth retardation and early senescence. Phenolic secretion was stimulated by cutting on the explant, thereby serving as a plant defence mechanism.

DISCUSSION

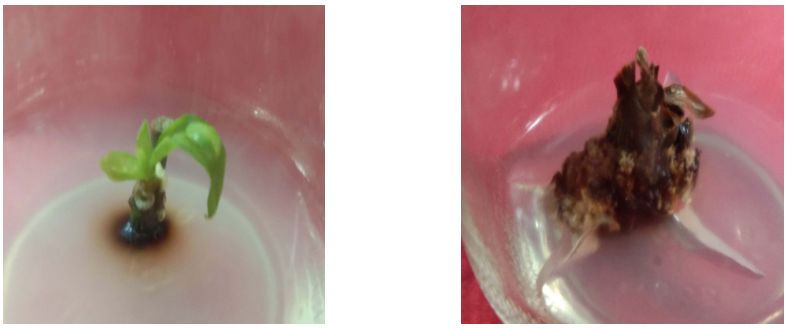
In recent years, pharmacologists have become concerned with the development of natural therapies that pose fewer or no negative effects on humans. Natural therapies are made of medicinal plant extracts, and these species contain certain phytochemicals that have the ability to maintain human health (Ekor, 2014). In general, persimmon could be considered

Table 3

Effect of different culture medium containing various plant growth regulator on persimmon shoot regeneration

Treatments	Leaves emergence (days)	Callus emergence (days)	Leaf number	Leaf length (mm)
Culture medium				
M1	52.11 a	38.67 a	1.11 a	3.21 a
M2	56.59 a	36.33 a	2.00 a	2.90 a
Plant growth Regulator				
Z1	54.38 p	46.50 p	1.50 p	3.07 p
Z2	54.56 p	36.00 p	2.00 p	3.55 p
Z3	54.12 p	30.00 p	1.17 p	2.55 p
Interaction		(-)		

Note. Means in each column followed by the same letter were not significantly different according to Duncan's multiple comparison test ($p \leq 0.05$). (-) no interaction between the two treatments



(a) (b)

Figure 4. Micropropagation of persimmon. (a) Leaves induction; and (b) necrosis plant tissue

as medicinal plant because of its promising phytochemical profile, and specifically, the antioxidant activity of persimmon (*D. kaki*) has been explored to cure various ailments (Butt et al., 2015). Persimmon has become a valuable species of Indonesian germplasms since its introduction one hundred years ago. While the distributed persimmons in Central Java and East Java are approximately 80 years old, fruit production is gradually decreasing because of improper cultural practices and environmental conditions. Therefore, conservation of this species has become crucial in order to preserve and further investigate its promising value.

As the existing persimmon population in Indonesia has not yet been characterized, genetic identification must be pursued in order to provide genetic information concerning this particular persimmon genotype. Nine selected RAPD primers successfully identified a genetic relationship among the persimmons; based on the similarity coefficient value, all of the persimmons were derived from the same genotype, with low genetic variation. Of note, the genetic variation of this persimmon was lower than that found in previous persimmon research (Akbulut et al., 2008; Badenes et al., 2003; Yamagishi et al., 2005; Yyldyz et al., 2007), possibly resulting from narrow persimmon distribution. Lower genetic variation commonly occurs in species with narrow geographical distributions because of genetic drift and inbreeding (Hamrick & Godt, 1990; Li et al., 2012; Willi et al., 2006). Genetic drift leads to heterozygosity reduction, while inbreeding increases homozygosity within populations. These factors have negative consequences on plant reproduction and survival rate (Ellstrand & Elam, 1993; Frankham, 2005). Genetic variation of persimmons has also been caused by plant stress adaptation. According to Boyko and Kovalchuk (2011), stress exposure induces DNA methylation, which consequently results in rearrangements of the genome. Such changes lead to different gene expressions that could be inherited by the progenies.

Using RAPD markers in the current study resulted in an efficient method for identifying this unknown persimmon genotype. Findings revealed that all of the persimmons were derived from same genotype; therefore, higher genetic variation of this persimmon is required to enhance species adaptation to changing environments for productivity improvement. Higher genetic variation could be obtained with the identification of other persimmon samples from other regions. According to Yamagishi et al. (2005), the application of long primer-RAPD (more than 10 base primers) enhance high reliability, reproducibility and polymorphism rate, resulting in a more accurate genetic identification.

Conservation of the persimmon genotype in Indonesia was continued through micropropagation in order to provide healthy and clonal seedlings. Mature lateral shoots were used to culture *in vitro*; the culture of mature tissue is preferred since desired traits at maturity could be determined (Rajeswari & Paliwal, 2007). Persimmon micropropagation was

successfully established using donor plants from Sened village. According to Benson (2000), the difference in regeneration ability also could be influenced by the different developmental stages of plants and by environmental factors. The mature explant is highly related to recalcitrance, a phenomena that refers to inability of plant cells to respond to the culture condition. The low regeneration of persimmon was also influenced by a high percentage of contamination due to a heavy rainy season during explant collection. Roussos and Pontikis (2001) have also reported that explants derived from field-growing trees had lower survival rates than explants derived from greenhouse-growing trees.

This study showed that there was no interaction between culture medium type containing various plant growth regulators toward explant regeneration, and that explant growth of each explant did not significantly differ. However, the highest mean of leaf number was achieved on WPM. The low salt concentration of WPM promotes plant cells regeneration by interacting with osmotic potential that increase water absorption from the medium to the cell (Beauzamy et al., 2015). WPM also gave better shoot length of Japanese persimmon compared to ½ MS. WPM containing BAP has been clarified as an optimum medium for the shoot growth of Japanese persimmon (Fukui et al., 1989). Besides, the highest mean of leaf length was achieved on the a half reduction of salt concentration in MS. The better results of persimmon shoot regeneration were obtained with ½ MS than with MS has been confirmed in previous research (Kochanova et al., 2011). The high salt concentration of culture medium causes lower stem thickening and higher vitrification (Araruna et al., 2017).

The result of this study also showed that plant growth regulator (IBA 2 ppm + BAP 2 ppm) also promoted the highest mean of leaf number and length. The individual application of BAP also successfully induces shoot regeneration of other persimmon cultivars (Belllini & Giordani, 1997; Fukui et al., 1989; Sarathchandra & Burch, 1991), while a combination of BAP and IBA tends to enhance persimmon shoot regeneration (Kochanova et al., 2011). The interaction of auxin and cytokinin has been confirmed for cell division, cell expansion and cell differentiation (Schaller et al., 2015; Su et al., 2011); BAP belongs to the adenine derivatives of the cytokinin class, which are known to be necessary for cell division. BAP also has the ability to induce reinvigoration of mature tissue and natural hormone production (Malik et al., 2005; Zhang et al., 2010). IBA is synthetic auxin that has been known for cell enlargement and as having the greatest stability for root formation (Frick & Strader, 2017; Nordström et al., 1991; Pasternak et al., 2000). The regeneration of this persimmon genotype was lower compared to other persimmon genotypes in the previous studies (Bellini & Giordani, 1997; Fukui et al., 1989, 1992; Kochanova et al., 2011; Palla & Beasley, 2013; Sarathchandra & Burch, 1991; Tao & Sugiura, 1992). This could happen because of different explant

types; different persimmon genotypes have different cell regeneration response to culture condition.

The formation of non-embryogenic callus is an undesired result because it has no organogenesis ability resulting in reduction of leaf number and length means. Callogenesis occurrence depends on genotype because of certain endogenous hormone levels that affect cellular totipotency. According to Jiménez and Bangerth (2001), non-embryogenic callus containing less endogenous indole-3-acetic acid (IAA), abscisic acid (ABA) and gibberellins (GAs), are unable to stimulate embryogenic competence. Callus differentiating ability is promoted by the addition of exogenous plant growth regulator. According to Mohajer et al. (2012), the addition of BAP and IBA successfully influences the balance of endogenous hormones, resulting in embryogenic callus formation of *Onobrychis sativa*. A higher percentage of callus formation (96%) in *O. sativa* was induced by BAP 2 ppm + IBA 3 ppm, while application of BAP 2 ppm + IBA 2 ppm induced 78% callus. The same BAP and IBA concentrations were applied on persimmon culture, but the non-embryogenic callus were highly induced. This different result may have been influenced by the recalcitrance of mature plant tissue. According to Fukui et al. (1989), culture medium containing cytokinin had significant effect on the callus formation at the base of persimmon shoot.

The growth retardation and necrosis of persimmon shoots were observed in this study; this was probably caused by browning. The negative effects of browning were also observed in other persimmon cultivars (Fukui et al. 1989; Sarathchandra & Burch, 1991). The high browning percentage (70%) is highly associated with high secondary metabolites contained in the persimmon (Miller & Murashige, 1976). Browning is triggered by mechanical damage and application of plant growth regulator (North et al., 2012). These factors regenerate reactive oxygen species (ROS) to activate stress-related gene expression (proteinase inhibitor, thionin and secondary metabolites) and hormones synthesis for callogenesis (Ikeuchi et al., 2017; Zhao et al., 2005). The accumulation of ROS causes lipid, protein and carbohydrate peroxidation, resulting in early senescence of plant tissues (Jajic et al., 2015).

Tissue browning is highly associated with increasing PPO activity followed by significant increase of phenolic compound production (Constabel et al., 2000; Thipyapong & Steffens, 1997). The oxidation of phenolic compound creates o-quinones that would be toxic for a plant by metabolism enzymes inactivation (Laukkanen et al., 1999). The deleterious effects of browning caused deterioration of callus regeneration and the insignificant growth of explants toward the culture condition. The results were supported by Chuanjun et al. (2015) that phenolic compound production interfered water and nutrition absorption by the vascular system, giving rise to growth retardation and gradual lethality to the plant.

Browning reduction treatment using ascorbic acid 10% was applied in this research, but the browning percentage was still high. According to Altunkaya and Gokmen (2008) and Nicholas et al. (1994), a higher concentration of ascorbic acid is required to convert the formed quinones into the diphenols and also to act as a PPO inhibitor. According to North et al. (2012), the high phenol content in the medium could be significantly reduced by 53% with the addition of activated charcoal (AC). The very fine pores, along with a large inner surface, of activated charcoal effectively absorb the inhibitory compounds and also promote cell growth (Thomas, 2008). The reduction of polyphenol production in persimmon has been observed earlier. The double layer system of MS containing 2% AC gave better shoots performance (Sarathchandra & Burch, 1991), while precultured persimmon shoot tips in a liquid medium exhibited better shoot growth (Fukui et al., 1989). Determining the most approriate treatment for browning reduction of this persimmon genotype is highly required in order to enhance explant regeneration.

Regeneration of this persimmon could be enhanced with aseptic culture modification. According to Purohit et al. (2011), CO_2-enriched environment, light intensity and modified ventilation promote a high photosynthetic rate and normal anatomical structure (thick cuticular wax, palisade cell, functional stomata), resulting in a higher success of *in vitro* regeneration and acclimatization rate. This modification facilitates low relative humidity, high gaseous exchange and a low ethylene level that enhance tissue vigour. Accelerating explant growth will shorten the plant life cycle, thus reducing the production cost associated with micropropagation.

A successful micropropagation at the initiation stage of the persimmon genotype in Indonesia was established. In this regard, the regenerated shoot must be subcultured on modified culture medium to induce persimmon shoot multiplication. The shoot proliferation rate of *Diospyros kaki* L., *D. kaki* Thun cv. 'Hiratanenashi' and 'Nishimurawase' was successfully induced by certain mediums containing BA or zeatin (Bellini & Giordani, 1997; Fukui et al., 1989; Palla & Beasley, 2013; Sarathchandra & Burch, 1991). Additionally, the multiplied shoots are needed to root in order to accelerate higher survival ability during acclimatization. Many persimmon cultivars have been successfully induced to root *in vitro* (Fukui et al., 1992; Palla & Beasley, 2013; Saratchchandra & Burch, 1991). These micropropagation stages must be attained if a large number of persimmon seedlings is to be provided. Further investigation is still required to determine appropriate treatments for each micropropagation stage of the specific persimmon genotype in Indonesia.

CONCLUSION

The genetic relationship of distributed persimmons in Central Java and East

Java provinces was identified by RAPD markers. Nine primers yielded a total of 32 polymorphic and 20 monomorphic bands. Based on the similarity coefficient value, all of the persimmons are derived from the same genotype. Regeneration of the genotype was conducted through micropropagation. Medium culture supplemented with various plant growth regulators did not significantly affect explant regeneration; the explant growth probably was limited by high browning percentage. As regenerated shoots could be used for further stages of persimmon micropropagation, additional investigation of browning reduction is also required in order to improve persimmon regeneration.

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