CHARACTERIZATION OF ZYGOTIC AND NUCELLAR EMBRYO OF SIX INDONESIAN MANGO CULTIVARS USING MOLECULAR MARKERS

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Abstract

One of difficulties in mangoes hybridization is polyembryonic seeds. This phenomenon reduces the chance of recovering true hybrid seedlings. The identification of the zygotic embryo is difficult and the possible approach is by using molecular marker. The objective of this study was to evaluate the utilization of two different marker systems (dominant markers and SSR) to characterize the occurrence of zygotic or nucellar embryo in polyembryony mango cultivars Garifta Merah, Lalijiwo, Manalagi, Madu, Saigon Kuning, and Saigon Merah. The type of embryo was evaluated by comparing the exhibiting amplification patterns, if different from the mother plant considered as zygotic and identified as nucellar if they exhibited the same banding pattern as the mother plant. From both of this marker systems out of the 16 SSR and 16 evaluated dominant primers, nine primers of each systems amplified the largest number of allele and sharply defined band. Dendogram analysis showed that the evaluated dominant markers could distinguish the zygotic and nucellar embryo clearly compare to evaluated SSR primers. The number of zygotic embryos derived from SSR was 64% and dominant markers were 47%. Based on zygotic and nucellar size, number and position, indicating no relationship between the type of embryo and embryo size, number and the position.

Key words: *Mangifera indica*, polyembryony, SSR, RAPD, SCoT, SAP

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Introduction

The genus *Mangifera* has about 70 members which are located on Malay Peninsula, in Indonesian, Thailand and Philippines (Bomphard, 2009). Domestication in the Indian region increased monoembryonic varieties while domestication in the Indochina, Thailand and Myanmar region (Southeast) including Indonesia increased polyembrionic varieties (Bompard and Schnell, 1997). The wide diversity of *Mangifera* genus occurred in Sumatera and Kalimantan (Iyer & Schnell, 2009). Diversity is the key point intended for breeding naturally or by man. It is required to have wide genetic pool to gain new combinations naturally or developing new varietes (Usman *et al*., 2001). However modern, well-organized breeding systems have not yet been developed for most tropical fruit species (Ogata *et al*., 2016).

Mango plants are propagated mainly by seed and grafting. Commercial varieties in Indonesia are Arumanis, Manalagi, Golek, Lalijiwo that are mostly produced in East Java and Gedong, Indramayu which are produced in West Java. Rebin and Karsinah (2010) reported that Garifta Merah was one of four varieties released in 2009 for export commodity with attractive red peel fruit color and a bit sour fruit taste. Madu variety was released in 2007 and has been used as rootstock for elite mango cultivar such as Arumanis, Golek 31, Manalagi 69, and Durih (Lalijiwo) in East Java province. Karsinah *et al*. (2015) reported that Saigon Kuning and Saigon Merah as new hope for rootstock variety delivered high percentage of grafting

viability and have similar performance with Madu.

Mango breeders are challenged by the polyembryony problem, the phenomenon of multiple seedlings (one zygotic seedling and several nucellar seedlings) emerging from a single seed. This characteristic reduces the possiblity of finding out the true hybrid seedlings (Schnell and Knight, 1992). In mango, the nucellar embryos are developed from the nucellar tissue that surrounding the embryo sac, and the seedlings descended from these embryos are genetically similar to the mother plant (Aron *et al.*, 1998). Nurserymen have used vigorous seedling for grafting that they are nucellar, which generate morphologically uniform plants. However, the zygotic embryo is derived from fertilization by self-pollination or by cross-pollination and the zygotic embryo is the objective in breeding programs for the selection of superior genotypes and desirable trait (Rocha *et al*., 2014).

Mango has been the subject of many analysis using different molecular-marker types as RAPD (Souza *et al*., 2011), SSR (Tasliah *et al*., 2013), SCoT and ISSR (Luo *et al*., 2011), CAPS (Shudo *et al*., 2013), EST-SSR (Dillon *et al*., 2014), SSR and SNP (Sherman *et al*., 2015). Because of the difficulty in selecting nucellar seedlings in seeds of polyembrionic mangos using morphological characteristics (Desai, 2004), the identification or differentiation of zygotic embryo among nucellar in polyembryonic seeds was examined by RAPD (Cordeiro *et al*., 2006; Ochoa *et al*., 2012), and ISSR (Rocha *et al*., 2014). The objective of this work was to evaluate the utilization of two different marker systems, i.e. dominant markers (RAPD, SCoT and SAP) and SSR marker to characterize the occurrence of zygotic or nucellar embryo in polyembryony mango cultivars Garifta Merah, Lalijiwo, Manalagi, Madu, Saigon Kuning, and Saigon Merah.

Materials and Methods

Plant Materials. The experimental material used in the present study consisted of 6 polyembryonic mango cultivars (Table 1). Young and healthy leaf samples (maternal) and fruits (embryo) were collected from the germplasm collections of Cukur Gondang field station of Indonesian Tropical Fruit Research Institute, Pasuruan, East Java. The endocarp and seed coat (testa with tegmen wrapping all embryos) were removed from each seed, and the number of embryos per seed was determined. The embryos were separated and numbered according to their position with respect to the funiculus (Figure 1). The embryo next to the point of insertion of seed funiculus in the seed coat was denominated 'one'; the rest were arranged clockwise and then numbered. The funiculus was used as the reference, since in anatropous ovules, such as mango, it is next to the micropyle (Bachelier & Endress, 2009).

Table 1. List of mango accession used in this study

No.	ID	Accession	Origin
1.	-	Garifta Merah	New Released Variety
2.	53-54	Lali Jiwo-61	Kraksaan, Probolinggo
3.	57-58	Madu-65	Pasuruan
4.	61-62	Manalagi-69	Pasuruan
5.	-	Saigon Merah	New Released Variety
6.	-	Saigon Kuning	New Released Variety

Figure 1. The seed and embryos performance of Saigon Kuning Variety. (A) The seed after the endocarp and seed coat were removed. (B) The embryos were separated and numbered according to their position with respect to the funiculus.

DNA Extraction. Leaf samples and embryos were collected. Genomic DNA extraction was performed following the method described by Yu (1994). The leaves tissue and embryos were grinded with mortar and pestle in liquid Nitrogen until they reached powder condition. Ice-cold extraction buffer (6,94 g Glucose 1 M, 2 g PVP, 10 ml Tris-HCl 1 M, 1 ml 0.5 M EDTA and 0.2 g Na disulphyt) was added and centrifuged at 3750 rpm for 20 minutes (4°C). The lysis buffer (2 g CTAB, 2 g PVP, 10 ml Tris-HCl 1 M, 1 ml EDTA 0.5M, 28 ml NaCl 5M) was added and vortexed to resuspend

pellet and incubated at 65°C water bath for 30 minutes. Chloroform: isoamyl alcohol (24:1) was added and centrifuged at 3750 rpm for 20 minutes (15°C). The upper phase was transferred into new tube and equal volume of cold isopropanol was added and centrifuged at 2500 rpm for 10 minutes (RT). The supernatant was discarded and cold 76% ethanol/0.2 M Na Acetate was added and centrifuged tubes (3750 rpm, 10 min). The pellet was rinsed with cold 70% EtOH and dissolved in TE buffer. To evaluate the DNA quality, electrophoresis in 0.8% (w/v) agarose gel was performed and dyed with ethidium bromide and photographed under ultraviolet light using the gel documentation system (BioRad). DNA concentration and purity was determined with a spectrophotometer at 260/280 nm absorbance readings.

Molecular Analysis. The PCR amplification was generated using Biorad Thermal Cycler PCR machine by following PCR conditions: (1) for SSR analysis used 16 primers (Duval *et al*., 2005; Schnell *et al*., 2006) (Table 2) (i) an initial denaturation step of 5 min at 94 ^{0}C, (ii) 30 cycles of 45 second at 94 ^{0}C, 45 second at 55 ^{0}C, 1 min at 72 ^{0}C and (iii) a final extension step for 5 min at 72^{0}C. (2) for dominant markers analysis used 16 primers (Cordeiro *et al*., 2006; Ochoa *et al*., 2012) (Table 3), 44 cycles of 1 min at 94 ^{0}C, 1 min at 35 ^{0}C, 2 min at 72 ^{0}C. Amplified products was separated by electrophoresis in 8% polyacrylamide gel (Dual Triple-Wide Mini-Vertical System, C.B.S. Scientific, CA, USA) for SSR analysis and Agarose gel for RAPD, SCoT and SAP followed by and observed by ethidium bromide and photographed under ultraviolet light using the gel documentation system

Table 2. List of SSR primers used in this study

No	Primer	Forward	Reverse
1	AY942818	Ccacgaatatcaactgctgcc	tctgacactgctcttccacc
2	AY942820	aggtcttttatcttcggccc	aaacgaaaaagcagccca
3	AY942822	caacttggcaacatagac	atacaggaatccagcttc
4	AY942829	gaacgagaaatcgggaac	gcagccattgaatacagag
5	AY942831	tttaccaagctagggtca	cactcttaaactattcaacca
6	AJ938175	gctctttccttgacctt	tcaaaatcgtgtcatttc
7	AJ938179	tcggtcatttacacctct	ttattgagcttctttgtgtt
8	AJ635168	ttctaaggagttctaaaatgc	ctcaagtccaacatacaatac
9	AJ635170	Gacccaacaaatccaa	actgtgcaaaccaaaag
10	AJ635171	taaagataagattgggaagag	cgtaagaagagcaaaggt
11	AJ635172	tagggatatagctggagg	acgcagtagaacctgtg
12	AJ635175	tgcgtaaagctgttgacta	tcatctccctcagaaca
13	AJ635180	Cctcaatctcactcaaca	accccacaatcaaactac
14	AJ635182	gacttgcagtttccttt	tcaagaaccccatttg
15	AJ635183	ccattctccatccaaa	tgcatagcagaaagaaga
16	AJ635187	atccccagtagctttgt	tgagagttggcagtgtt

Data Analysis. The SSR analysis was based on the alleles sizes generated and implemented in PowerMarker V3.25 (Liu & Muse 2005) that calculated the allele frequency and polymorphism information content (PIC). The dominant markers analysis was scored as coded in binary form 1 or 0, respectively. The polymorphic information content (PIC) for dominant marker according to Powell *et al*. (1996): PIC = 2*f*i (1-*f*i). The UPGMA method (unweighted pair group method arithmetic average) was used to construct a dendrogram using NTSYSpc 2.11p software (Rohlf, 2005). Plants exhibiting amplification patterns different from the mother plant were considered zygotic. Amplified products were recognized as polymorphic based on the presence or absence, in the different samples, without considering differences of intensity. In this SSR analysis only for five cultivars (without Manalagi) that pursued for analysis.

Table 3. List of dominant markers used in this study

No.	Primer	Sequence
1	OPE6	Aagacccctc
2	OPF3	Cctgatcacc
3	OPF7	Ccgatatccc
4	OPG6	Gtgcctaacc
5	OPG13	Ctctccgcca
6	OPG19	Gtcagggcaa
7	OPH4	Ggaagtcgcc
8	SCoT61	caacaatggctaccaccg
9	SAP 1	atg cgaacc g
10	SAP 2	gac aca tcg g
11	SAP 3	tgg gac ctc c
12	SAP 4	gga gct acct
13	SAP 5	tat agg ccc t
14	SAP 6	cctact cca g
15	SAP 7	tgg gaa tcc c
16	SAP 8	gcc cct act a

Results

SSR Analysis. A total of 65 alleles, with an average of 4 alleles per locus were detected at the 16 SSR loci (Figure 2). The number of alleles per locus ranged from 2-9 with the allele size ranged from 50-450 bp. Polymorphism Information Content (PIC) values, a reflection of allele diversity and frequency among the varieties, averaging 0.48 and ranging from a low of 0.07 (AJ938175) to a high of 0.84 (AJ635171) while AJ938175 was present the highest frequency allele up to 96% and AJ635171 showed the lowest frequency alleles 23% (Table 4). Out of the 16 SSR primers evaluated, 9 primers amplified

the largest number of allele and sharply defined band (4 to 9 bands): AJ635170, AJ635183, AJ635172, AJ938179, AY942831, AJ635175, AJ635168, AJ635187, and AJ635171 (Table 4).

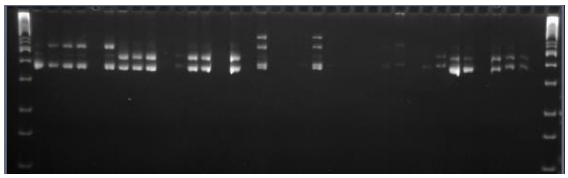

Figure 2. DNA banding pattern of amplified PCR product of AY942829 primer in 8% acrylamide gel electrophoresis. Lane 1 (from left to right): Marker 100 bp, Garifta Merah (M, E1, E2,E3, E4, E5), Lalijiwo (E1, E2, E3, E4, E5), Saigon Kuning (M,E1a,E1b,E2,E3,E4,E5,E6,E7,E8), and Madu (M,E1,E2,E3,E4,E5,E6,E7,E8,E9).

Table 4. Summary statistic of SSR primers.

Primer Name	Major allele frequency	Allele number	Band size (bp)	PIC
AJ938179	0.43	5	210-300	0.65
AY942822	0.5	2	200-280	0.38
AY942831	0.47	5	50-300	0.65
AY942829	0.48	3	370-450	0.49
AJ635171	0.23	9	50-280	0.84
AJ635170	0.6	4	160-250	0.51
AY942818	0.86	2	60-150	0.21
AJ635183	0.7	4	50-230	0.42
AJ635172	0.52	4	300-410	0.63
AJ635182	0.35	3	50-170	0.59
AJ938175	0.96	3	50-200	0.07
AJ635180	0.69	3	100-270	0.36
AJ635175	0.76	5	50-360	0.37
AY942820	0.82	2	50-230	0.25
AJ635168	0.59	5	50-200	0.56
AJ635187	0.32	6	70-320	0.67
Average	0.58	4	50-450	0.48

The Dendogram of 16 SSR primers (r=0.90) described the materna and embryos of five polyembrionic mango cultivar revealed three groups (Figure 3). In this study, the SSR primers could not distinguish sharply between groups into zygotic or nucellar. However it might be considered as the first group consist of Materna (M) of Garifta Merah, Saigon Kuning, Madu, Saigon Merah and Lalijiwo and several embryos of those cultivars and the second group consist of Saigon Kuning and Madu embryos.

Dominant Markers Analysis. A total of 126 fragments, with an average of 7,9 fragments per locus were detected at the 16 loci (Figure 4). The number of fragments per locus ranged from 1-15 with the fragment size ranged from 100-2000 bp. The PIC values, averaging 0.44 and ranging from a low of 0.25 (SAP2) to a high of 0.50 (OPG13 and SAP5) while SCoT61 and SAP1 were present the highest frequency fragments up to 65% and 64% respectively and SAP2 showed the lowest frequency fragments 15% (Table 5). Out of the 16 evaluated dominant primers, 9 primers amplified the largest number of sharply defined bands (8 to 15 bands): OPE-6, OPF-3, OPF-7, OPG-6, OPG-13, OPG-19, OPH-4, SAP-3, and SAP-4 (Figure 4).

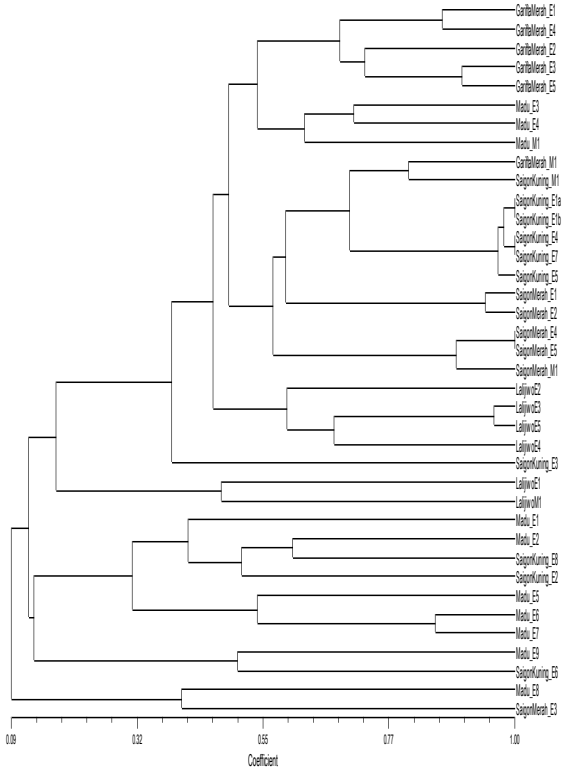

Figure 3. Dendogram of polyembryonic mango; zygotic and nucellar/maternal (M) obtained from five mango cultivars based on UPGMA clustering method of 16 SSR primers.

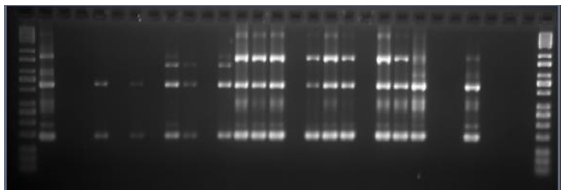

Figure 4. DNA banding pattern of amplified PCR product of OPE6 primer in 2% agarose gel. Lane 1 (from left to right): Marker 100 bp, Garifta Merah (M, E1, E2,E3, E4, E5), Lalijiwo (E1, E2, E3, E4, E5), Saigon Kuning (M,E1a,E1b,E2,E3,E4,E5,E6,E7,E8), and Madu (M,E1,E2,E3,E4,E5,E6,E7).

Table 5. Summary statistic of RAPD, SCoT and SAP primers.

Primer Name	The number of Fragment	Fragment frequency	Band size (bp)	PIC
OPE6	8	0,41	300-1800	0,48
OPF3	9	0,33	200-1300	0,44
OPF7	15	0,25	300-2000	0,38
OPG6	11	0,34	250-2000	0,45
OPG13	11	0,48	200-2000	0,50
OPG19	15	0,34	70-2000	0,45
OPH4	11	0,41	350-2000	0,48
SCoT61	4	0,65	400-1600	0,45
SAP 1	1	0,64	400	0,46
SAP 2	5	0,15	600-2000	0,25
SAP 3	10	0,39	200-2000	0,48
SAP 4	8	0,26	300-2000	0,38
SAP 5	3	0,51	400-600	0,50
SAP 6	5	0,39	100-1800	0,47
SAP 7	5	0,31	500-1500	0,43
SAP 8	5	0,28	200-1200	0,41
average	7,9	0,38	100-2000	0,44

The materna and embryos of the six polyembrionic mango cultivar formed two major groups described in the dendogram based on 16 evaluated dominant primers (r=0.94) can be clearly observed (Figure 5). The first group (Materna group) consist of Materna (M) of Garifta Merah, Saigon Kuning, Madu, Manalagi, and Lalijiwo and several embryos of those cultivars within this group were identified as nucellar as they were grouped together with their materna. The second group (Zygotic group) consist of several embryos of the five cultivars which their materna located in the first group however the materna of Saigon Merah was grouped in the second group while its embryo in the first group considered as zygotic.

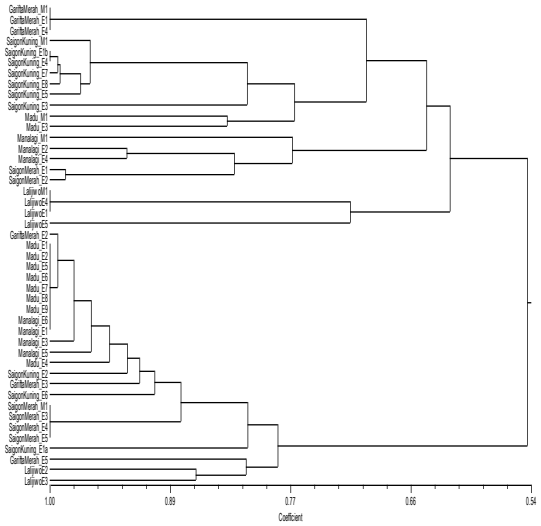

Figure 5. Dendogram of zygotic and nucellar embryos obtained from six mango cultivars using UPGMA clustering method of 16 dominant primers.

Identification of Embryo Type. The type of embryo (zygotic or nucellar) was evaluated by comparing the exhibiting of amplification patterns. Zygotic was identified as if different from the mother plant and nucellar as if they exhibited the same banding pattern as the mother plant. In SSR primers, the embryos of Garifta Merah (E1, E2, E3, E4, and E5), Saigon Kuning (E2, E3, E6 and E8), Saigon Merah (E3), Lalijiwo (E2, E3, E4 and E5), and Madu (E1, E2, E5, E6, E7, E8, E9) were identified as zygotic while in dominant primers the embryos of Garifta Merah (E2, E3 and E5), Saigon Kuning (E1a, E2 and E6), Saigon Merah (E1 and E2), Lalijiwo (E2, and E3), Madu (E1,E2,E4,E5,E6,E7,E8,E9) and Manalagi (E1, E3, E5 and E6) were identified as zygotic. The percentage of zygotic embryos from six evaluated polyembryonic Indonesian mangoes cultivars derived from SSR marker analysis revealed 64% zygotic while from the evaluated dominant markers was 47% (Table 6). However no single SSR or dominant primers by itself could identify all the zygotic embryos. Therefore the set of those nine primers together could detect the zygotic/nucellar embryos of those cultivars.

Table 6. Summary of zygotic embryo based on SSR and dominant markers (RAPD, SCoT and SAP) analysis

Mango Cultivar	Embryo per seed	SSR Analysis	
		Materna/ Nucellar	Zygotic
Garifta Merah	E1-E5	-	E1,E2, E3,E4,E5
Saigon Kuning	E1-E8	E1a, E1b, E4,E5,E7	E2,E3,E6,E8
Saigon Merah	E1-E5	E1, E2, E4, E5	E3
Lalijiwo	E1-E5	E1	E2, E3, E4, E5
Madu	E1-E9	E3, E4	E1,E2, E5, E6,E7,E8,E9
Mango Cultivar	Embryo per seed	Dominant markers Analysis	
		Materna/ Nucellar	Zygotic
Garifta Merah	E1-E5	E1, E4	E2, E3, E5
Saigon Kuning	E1-E8	E1b, E3, E4, E5,E7,E8	E1a, E2, E6
Saigon Merah	E1-E5	E3,E4,E5	E1, E2
Lalijiwo	E1-E5	E1, E4,E5	E2, E3
Madu	E1-E9	E3	E1,E2,E4,E5,E6, E7,E8,E9
Manalagi	E1-E6	E2,E4	E1,E3, E5,E6

Discussion

The chance for breeding improvement in mango is challenging. The multiple seedlings phenomenon in breeding program can be resolved by using marker-assisted selection (MAS) as useful tools for genotype selection of economically important crops. Molecular markers can be used to identify true hybrids in polyembryonic species such as Indochinese type mango and some citrus species (Oliveira *et al*., 2002). Furthermore, MAS allows early selections of major genes controling traits and thus reduces the time and space needed for growing out seedlings (Chunwongse *et al*., 2015).

The efficiency of a marker system in discriminating genotype depends on polymorphism it can detect (Guo *et al*., 2014). In this study, both marker systems (dominant and SSR) were used for evaluating the zygotic or nucellar embryos. It was observed that both marker systems giving different outcomes in distinguishing between the zygotic and nucellar embryos (Figure 3 & Figure 4). The discrepancy between both marker systems might be due to the differences in the source of detected polymorphism. Each system targets different regions of the genome (Gorji *et al*., 2011). In this study dominant markers consisted of RAPD, SCoT and SAP primers that use a single primer as the forward and reverse primers differ from SSR primers that use both forward and reverse primers (Collard *et al*., 2009).

Furthermore, the PIC value can be determined based on the number and frequency of amplified fragments to measure the discriminatory power of a genetic marker system (Roldan *et al*., 2000). In this study, the average PIC value of the evaluated SSR primers (Table 4) was higher than the PIC of dominant markers (Table 5) however in fact it was lower than determined PIC value for SSR system (maximum PIC value for co-dominat marker is 1.0) therefore it could not differentiate sharply while in dominant marker system, its PIC value of the evaluated dominant markers was high. The PIC value still confirmed the good discriminatory capacity of the primers as a maximum PIC values of 0.5 for dominant markers (De Riek *et al*., 2001). As the result, the dominant markers systems could differentiate between zygotic and nucellar embryos clearly. Rocha *et al*. (2014) reported the use of ISSR marker (dominant marker) as tool for the identification of the genetic origin of seedlings from 'Ubá' mango tree seeds.

Taken to be account that RAPD, SCoT and SAP primer are dominant marker therefore it could not identify the zygotic seedlings in homozygote or heterozygote condition that preferred for breeding program, while codominant marker could be used to confirm the parentage of F1 progenies during the breeding process.

In this study, the percentage of zygotic embryos from six evaluated polyembryonic Indonesian mangoes cultivars was 47-64%. This result was in line with Schnell *et al.* (1994) reported that up to 66% of zygotic off types in different varieties of polyembryonic mango. The lowest number of zygotic embryos (higher number of nucellar/maternal embryos) was Saigon Kuning and Saigon Merah (~30%). In parallel with Karsinah *et al*. (2015) that reported Saigon Kuning and Saigon Merah as rootstock variety for delivered high percentage of grafting viability. Kishor and Usha (2013) explained that nucellar seedling preferred for grafting, because they maintain the same genetic background of the rootstock mother-plant.

In agreement with Ochoa *et al.* (2012), in this study, more than one zygotic embryos per seed were found, they were very similar genetically (Figure 3 & Figure 5). These results suggest the chance of fertilization by different microgametes, as reported by Filho *et al.* (1993) for citrus. Aleza *et al.* (2010) mentioned that apomictic processes are referred to as polyembryonic and nonapomictic processes as monoembryonic.

Cordeiro *et al*. (2006) reported that regarding the position of the zygotic embryo, it is expected to be the first position, the embryo next to the point of insertion of seed funiculus in the seed coat. This position probably is the one in which favors the fecundation phenomenon in the embryo sac. However, in this study it was observed that the zygotic embryo can be found also in the second, third, fourth, fifth and sixth position.

The embryo size is an important characteristic in polyembryony, since smaller ones are generally not viable, lacking sufficient food reserves, or because they dehydrate in mature seeds (Andrade‐Rodríguez *et al.*, 2005). In the

present study, the larger size of embryo (E1, E2, E3 and E4) and the small embryo (E5, E6, E7, E8, E9) both could be identified as zygotic embryo. Villegas and Andrade (2008) reported that, in orange, the size of the embryos decreases as their position nears the micropyle tip of the seed. Andrade‐Rodríguez *et al*. (2004) reported in citrus, the larger the number of embryos per seed, the smaller the size of all embryos. This was not observed in the present study, in which only the size and position of embryo that was affected by the number of embryos per seed.

Mango breeders are challanged with severe yield loss due to premature fruit drop and fruitlet abscission as natural process (Guzman-Estrada, 1996). Premature fruit drop can be caused by several factors, one of them is lack of pollination or embryonic degeneration (Lovatt, 1997). This study, is the preliminary study in mangoes breeding in Indonesia to assist breeders to confirm their premature fruit drop after they conducted embryo rescue treatment in tissue culture laboratory. The plantlet originated from the polyembryonic seed could be validated by molecular markers used in this study to differentiate the zygote and nucellar plantlet.

It could be concluded from this study using two marker systems that out of the 16 SSR and 16 evaluated dominant primers, nine primers of SSR: AJ635170, AJ635183, AJ635172, AJ938179, AY942831, AJ635175, AJ635168, AJ635187, and AJ635171 and nine primers of dominant marker: OPE-6, OPF-3, OPF-7, OPG-6, OPG-13, OPG-19, OPH-4, SAP-3, and SAP-4 amplified the largest number of allele and sharply defined band and based on dendogram analysis, the evaluated dominant markers could distinguish the zygotic and nucellar embryo clearly compare with evaluated SSR primers. The percentage of zygotic embryos from six evaluated polyembryonic Indonesian mangoes cultivars derived from SSR marker analysis revealed 64% zygotic while in dominant markers was 47%. Based on zygotic and nucellar size, number and position, indicating no relationship between the type of embryo and embryo size, number and the position.

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