RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
51 (2) 151-160, 2019

THE ABSOLUTE GENETIC INVARIABILITY OF POLYEMBRYONY SEEDLING OF DUKU 'KUMPEH' (*Lansium parasiticum* (Osbeck) K.C. Sahni & Bennet.), A SUPERIOR TYPE FROM JAMBI, INDONESIA

P. MURNI[1], SYAMSUARDI[2*], NURAINAS[2], E. MANSYAH[3] and M. HUDA[2]

[1]Department of Biology, Faculty of Education, Universitas Jambi, Indonesia
[2]Departement of Biology, Faculty of Mathematics & Natural Sciences, Universitas Andalas, Indonesia
[3]Indonesian Tropical Fruit Research Institute, Solok, West Sumatra, Indonesia
*Corresponding author's email: syamsuardi@sci.unand.ac.id
Email addresses of coauthors: pintasyifa@gmail.com, nas_herb@yahoo.com, ellina_mansyah@yahoo.co.id, mihudahuda@gmail.com

SUMMARY

Duku 'Kumpeh' is one of the superior and potential cultivar of Duku (*Lansium parasiticum*) that originate from a specific area in the Jambi and it becomes one of the essential sources for income of Jambi people. For sustainable utilization of this essential local germplasm of tropical fruit need the understanding of the phenomenon of polyembryony and genetic information of the plant. The study of genetic variation of multiple seedlings of duku from Kumpeh was conducted. In this study, 34 seedlings were used (14 seedlings from Kumpeh and 20 seedlings from Dusun Tuo) from two mother trees in order to collect the samples of DNA. Extracted DNA samples used a modification of CTAB method then amplified using four primers of Inter-Simple Sequence Repeat (ISSR) namely $(AAC)_6$, $(AAG)_6$, $(AAT)_{10}$, $(AG)_{10}$ and two from RAPD markers (OPA 13 and OPA 18) to asses genetic variation. Base on observed and analysis of genetic variation of samples showed that multiple seedlings of seven seeds (14 seedling individuals) from Kumpeh and ten seeds (14 seedling individuals from Dusun Tuo) have the uniform of DNA band profile and the same as those of mother trees. The existing identical of all seedlings suggested that polyembryony of duku seeds derived from the non-zygotic embryo. Thus, we concluded that the reproduction system of duku (*Lansium parasiticum* (Osbeck) K.C. Sahni and Bennet.) occurred asexual with apomictic reproduction.

Key words: *Lansium parasiticum*, genetic uniformity, polyembryony, non-zygotic embryo

Key findings: The polyembryony of duku was a potential mechanism to produce a high number of seedlings with good quality and their genetic uniformity. The existing identical of all seedlings from polyembryony suggested the embryo derived from the non-zygotic embryo. The reproduction system of duku (*Lansium* *parasiticum* (Osbeck) K.C. Sahni and Bennet.) was asexual reproduction (apomictic).

Manuscript received: February 24, 2019; Decision on manuscript: March 7, 2019; Accepted: March 30, 2019.

Communicating Editor: Prof. Naqib Ullah Khan

INTRODUCTION

Duku (*Lansium parasiticum)* is a tropical fruit tree species belongs to the family of Meliaceae. The distribution of this species in South-East Asia mainly Peninsular Thailand, Malesia, Indonesia especially Sumatra and Java, Borneo, and Malay Peninsula (Mabberley *et al.,* 1995), but this species was distributed until Kuba, Honduras, India, Puerto Rico, Suriname, Thailand, Trinidad, Tobago, Amerika and Vietnam (Orwa *et al.,* 2009).

Duku 'Kumpeh' is one of the superior and potential cultivars of Duku (*Lansium parasiticum* (Osbeck) K.C. Sahni and Bennet.) that originate from a specific area in the Jambi, namely Kumpeh village and it become one of an essential source for income of Jambi people. Until now, commonly the local people have still produce the seedlings from the seeds that why the time for harvesting was longer. However, some cultivation from a vegetative organ such as cuttings, and grafts were not a success due to the problems of the thin of stem bark and the difficulty to remove from the wood. The seedling from polyembryony of duku (Salma and Razali, 1987) was an alternative way to produce multiple seedlings from a single seed during germination. Polyembryony plays a vital role in horticulture and plant breeding (Kishore, 2014). About 255 genera belonging to 153 families of Angiosperm plants were reported to exhibit the polyembryony (Carman, 1997). However, the seedlings from polyembryony of each plant species indicated the genetic variability between them.

Generally, the seedlings of duku resulted from germination, and 25% of those seedlings resulted from polyembryony (Salma and Razali, 1987). So it means the utilization of polyembryony to produce seedlings of duku still limited and application polyembryony to obtain the more seedlings is prospective for duku plantation. However, the seedlings resulted from polyembryony indicate genetic variability due to the zygotic process. In order to produce a good quality of this tropical fruit, the understanding of genetic uniformity is fundamental.

Some seedlings from polyembryony were resulted from zygotic and non-zygotic embryo process and will affect their genetic uniformity. For sustainable utilization of this essential local germplasm of tropical fruit, the information about genetic uniformity of seedlings from polyembryonic was crucial. The purpose of this study was to detect the level of genetic uniformity of duku seedlings as resulted from a specific pattern of polyembryony of a potential type of duku from Kumpeh, Jambi.

MATERIALS AND METHODS

This study used the samples of seeds collected from two mother trees in Kumpeh (KP) and Dusun Tuo (TB) during a fruiting season in 2016 (Figure 1). The position site is at S: 01°34.254′- E: 103°51.350′ Kumpeh and at S: 01°23.112′- E: 102°20.922′ Dusun Tuo, Jambi province Indonesia (Murni *et al*., 2016). Totally seventeen seeds (seven seeds from the individual tree of Kumpeh and ten seeds from the individual tree of Dusun Tuo were germinated in the greenhouse to obtain thirty-four seedlings of duku. Based on their size, seedlings by Kishore (2014) classified into two types. Initial individuals emergence with more significant big presumed as a zygotic seedling (hereafter mentioned by A-Type), in case of Individuals the subsequent emergence with smaller in size presumed as non-zygotic seedlings (B-Type).

Figure 1. The collection site of two mother trees from Kumpeh and Dusun Tuo (Tebo), Jambi Province, Indonesia (Modification from *http://www.google.com/maps/place/Jambi, Indonesia*).

The young leaves of those seedlings were extracted using CTAB (cetyl trimethyl ammonium bromide) method to obtain genomic DNA of progenies (Doyle and Doyle, 1990). About 0.1 mg fresh young leaves was ground for total DNA extraction by addition 1% of polyvinylpyrrolidone (PVP). The DNA concentration was measured by subjecting the samples into 1.2% agarose gel electrophoresis, staining with ethidium bromide and visualization on ultraviolet (UV) transilluminator used Bio-Doc Analyser (BDA) Biometra.

The simple, rapid and multiple polymorphic loci ISSR and RAPD markers were applied in this study. The four polymorphic ISSR primers [$(AAC)_6$, $(AAG)_6$, $(AAT)_{10}$, and $(AG)_{10}$] (Murni *et al*., 2016) and two polymorphic RAPD primers, OPA-13 (5'CAG CAC CCA C3') and OPA 18 (5' AGG TCA CCG T 3') (Yulita, 2011) were used in this study. PCR amplification of DNA samples using ISSR and RAPD primers conducted with a total volume of 12.5 ml solution containing 6.25 mL of Taq Go Green Master Mix Promega, 4.25 mL of dd H_2O (Nuclease-Free Water), 1 mL of primer, and 1 mL of sample DNA (template), Amplification using PCR thermocycler, GSX1 nexus Eppendorf Master cycler with 45 cycles. The reactions was initially with denaturation step at 95°C for one minute, a denaturation step at 95°C for one minute, annealing at temperatures appropriate optimization for one minute, extension at 72°C for two minutes, and a final extension at 72°C for 10 minutes. The results of PCR amplification subjected to electrophoresis on a 1.2% agarose gel immersed in TBE buffer and running with 110 V. Furthermore, applied electrophoresis gel was stained with 1% Ethidium bromide for 10 minutes and washed by soaking in distilled water for 15 minutes. Finally viewed under ultraviolet light and photographed using BDA Biometra.

Data analysis

The difference frequency of the number of seedling per seed of Duku Kumpeh between the two collection sites was statistically analyzed using the Mann-Whitney U Test on the degree of 95% confidence. The profile of DNA from all seedlings observed and compared between A and B type from different mother tree. The seedlings were assumed as non-zygotic embryo if the band pattern of the progeny was no different to those patterns of bands the mother tree. The differences pattern of the bands will be analyzed and the number of bands per primer, the polymorphic and monomorphic band was identified. The banding pattern between the seedling of progeny and mother tree were compared.

The bands of progeny and mother trees were scored, present of band = 1 and no detected of band = 0. The pattern of banded was arranged as a biner data and analyzed to produce the degree of genetic variation using POPGENE version 1.32. The genetic distance between progeny and the mother tree was calculated with the Jaccard similarity index (Yeh *et al*., 1997).

RESULTS

Thirty-four seedling from seventeen seeds (seven seeds from Kumpeh and ten seeds from Tebo) produced one to six embryos. Based on the number of an embryo produced from two collection sites, the frequency number of seedling were statistically different between Kumpeh and Dusun Tuo (Mann-Whitney $P < 0.01$). Duku from Kumpeh had a higher percentage of polyembryony (2-6 embryo) than duku from Dusun Tuo (Figure 2a), the percentage of the monoembryo was found more in duku from Dusun Tuo. Based on this, the duku seeds from Kumpeh more able to produce polyembryony seeds.

The observation of seedling performance from germination of 17 seeds indicated that there were

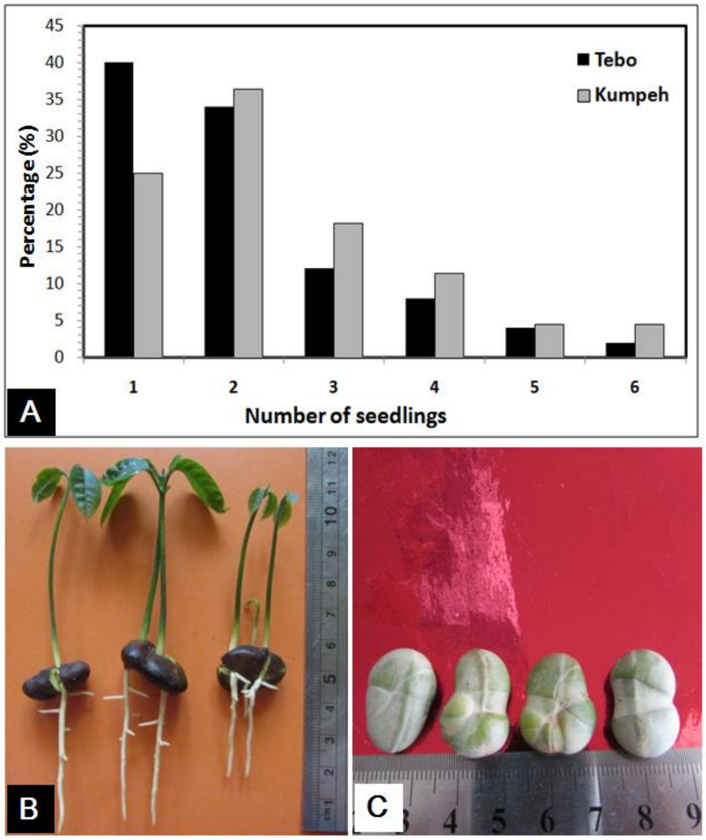

Figure 2. The variation of seedlings production from polyembryony seeds of duku in two collection site (A); The performance of seedlings from the different number of seeds (B); Morphological of some seeds with the different number of cotyledons (C).

variations in the growth of monoembryo and polyembryony seedlings. Seedling with one embryo tends to have higher and more significant growth compared to polyembryony. Diembryo was somewhat similar to monoembryo growth (Figure 2b), triembryo had a smaller stem growth, while 4-6 embryo showed stem and leaf curling and did not develop. Therefore, only mono embryo and diembryo were the potential to become a seedling. The Morphology of multiples seedling were different between the two types. The prior emerged seedlings were higher and more significant than those the next emerged seedlings. However, about five months after plantations, their performance of growth was relatively similar. The similar results also were reported by Irianto (2012), where most of the seedlings were with normal growth after six months.

DNA samples extracted from 34 young leaves of the seedlings. That DNA extraction obtained from seven A-type seedlings and ten B-type seedlings of Kumpeh, and seven A-type seedlings and ten B-type seedlings of Dusun Tuo. From analyzing the molecular data based on 34 seedlings of 17 seeds were carried out, used four selected primers for

Table 1. The repeated pattern of primers inter simple sequences repeat (ISSR) and RAPD, bands number and nucleotide size amplified DNA.

Primers	Sequences	Number of Bands	Nucletide Size (bp)
HQ200182	(AAG)6	7	1600; 1310; 960; 820; 720; 570; 450
HQ200181	(AAC)6	6	5290; 1940; 1360; 1070; 640; 410
HQ200186	(AAT)10	4	2830; 2000; 1180; 930
DQ453906	(AG)10	4	1060; 920; 740; 450
OPA 13	5’CAGCACCCAC3’	7	1500; 1190; 950; 860; 670; 530; 440
OPA 18	5’AGGTCACCGT3’	3	1150; 890; 590

ISSR and two RAPD primers indicated there was variation number of bands per primer. Twenty-eight bands resulted from analysis with seven bands detected to a primer $(AAG)_6$ and OPA-13 respectively, six bands at primer $(AAC)_6$, four bands at $(AAT)_{10}$ and $(AG)_{10}$ respectively. The nucleotide size of each band revealed in Table 1.

In general, most individuals resulted from the polyembryony mechanism were similar genetically, but in some cases, a few individual plants have different genetically due to gene flow from other individuals. Base on examination of twenty-one loci at ISSR and seven loci RAPD resulted from all of the progenies (polyembryony: seedling A and B) both from Kumpeh, and Dusun Tuo possessed the identical genetic pattern to their mother trees with the average of Jaccard Similarity = 1.0. Selected examples of identical genetic pattern amplified with ISSR primer $(AAT)_{10}$ and $(AG)_{10}$ and RAPD primer OPA-18 were shown in Figures 3 and 4, respectively. This fact suggested that all individuals in both the population and between populations tested have a 100% resemblance.

DISCUSSION

The assessment of fruit quality and performance requires time. The poor farmers usually compelled to wait for long period to understand the performance of the plants. They usually keep the uniforms population generated from a single selected clone and after some years rogue excess plants. Till the screening of exact clone they unnecessarily spent enormous resources on the rearing of excess seedlings. To reduce their financial investment, an early screening of the seedlings is necessary (Mondal *et al.,* 2014).

We hence suggest that polyembryony can serve as a counter-strategy by the mother against brood reduction driven by sibling rivalry. Brood reduction at early stages of offspring development saves the maternal parent considerable amounts of resources. By investing such saved resources in the nucellar embryos along with the surviving sexual embryo, the maternal parent can make good her fitness loss due to brood reduction.

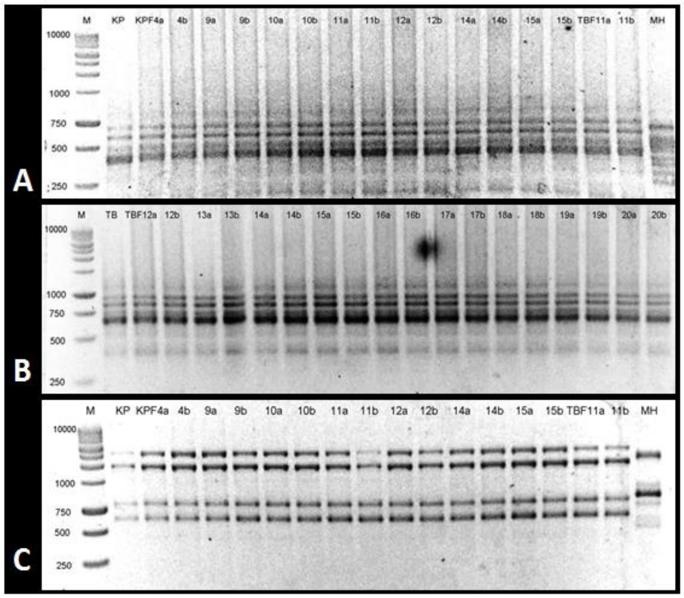

Figure 3. The similar pattern of bands from two mother trees and their progenies polyembryony in Kumpeh and Tebo resulted from amplification of ISSR marker $(AG)_{10}$ (A and B) and $(AAT)_{10}$ (represented by Kumpeh) (C); M = marker, KP and TB = mother tree of Kumpeh and Tebo, KPF and TBF = progenies of Kumpeh and Tebo, a and b = multiple seedlings from each seed, and MH = *Swietenia mahagoni* as the comparative group.

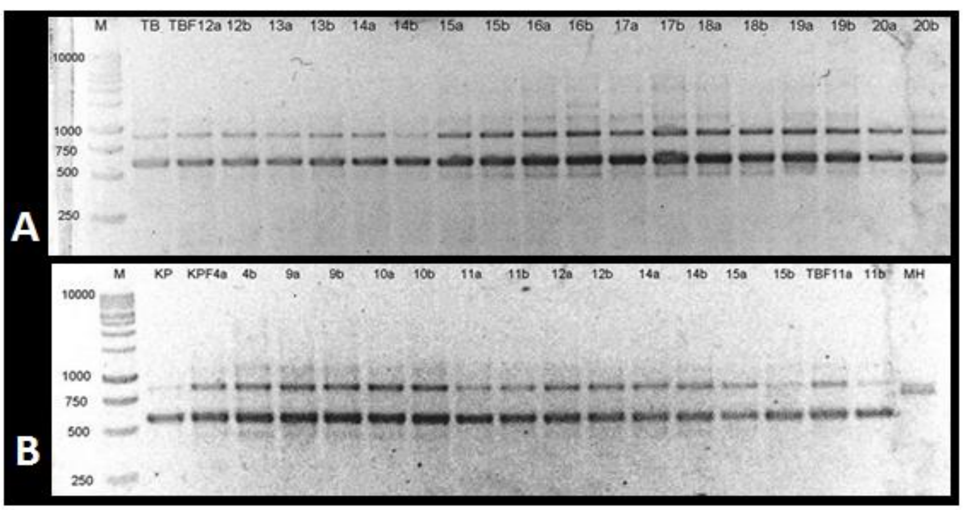

Figure 4. Similar pattern of RAPD bands from two mother trees and their progenies polyembryony in Tebo (A) and Kumpeh (except TBF11a, 11b) (B) resulted from amplification of RAPD marker OPA 18,M = marker, KP and TB = mother tree of Kumpeh and Tebo, KPF and TBF = progenies of Kumpeh and Tebo, a and b = multiple seedling from each seed, and MH = *Swietenia mahagoni* as the comparative group.

Quite a lot of the genera of angiosperms experience polyembryony so the potential to produce germplasm in many quantities, this aspect is significant in horticulture. In the case of Duku, the polyembryony was detected in Duku from Jerangau and Terengganu in Peninsular Malaysia with 1-4 embryo (Salma and Rozali, 1987). Hence, the distribution and environment factor affected the number of polyembryony. They produce a considerable number of ovules and number of seeds set in fruit provides the extent of brood reduction. It presumed that polyembryony is a maternal counter strategy to overcome the loss in her fitness due to brood reduction. So, the average number of polyembryony per seed should be negatively related to the seed number per fruit, i.e., a smaller number of seeds imply a higher level of brood reduction. A greater extent of polyembryony should counter this situation, obtained by producing more nucellar embryos.

Polyembryonic varieties produce seeds which originate zygotic and nucellar plantlets. Zygotic plantlets are sexual whereas nucellar plantlets come from the maternal tissues which are asexual plantlets. Therefore, nucellar plantlets are preferred for grafting, because they maintain the same genetic background of the rootstock mother-plant whereas zygotic plantlets preferred for breeding programs because they do not maintain same genetic background of the mother plant (Kumar *et al.*, 2013).

Usually the pattern of band produced from amplification ISSR and RAPD markers were polymorphic among samples examined (Parab and Kirshnan, 2008). However, DNA band profile resulted from amplification of ISSR and RAPD primer was monomorphic of each primer for all examined samples. All seedlings were very similar (identical) to those patterns of the mother tree (similarity index = 1, and genetic distance value = 0). The data revealed that the seedlings not derived from the fertilization of gamete cells, so it presumed that there was no gene flow between mother trees, and produced seedling only involved a mother tree, thus produced seedlings were same genotypes (Kumar *et al.*, 2013). Therefore, we presumed they were uniparental inheritance. Observation of pollens showed that there was no pollen was detected in the anthers of duku flowers. This evidence supported the assumption that fertilization did not occur during the reproductive period, and embryos resulted from the non-zygotic processed and derived from gametophytic or sporophytic (Batygina and Vinogradova, 2007).

In polyembryonic seed formation, many non-zygotic, nucellar embryos are initiated directly from the maternal, nucellar cells surrounding the embryo sac containing a developing zygotic embryo. During embryo sac expansion, embryogenic nucellar cells obtain access to endosperm and develop into embryos alongside the zygotic embryo that may or may not complete development. Nucellar embryos give rise to seedlings that are of the same genotype as the female parent (Koltunow *et al*., 1996).

CONCLUSION

Based on genetic variation analysis of multiple seedlings of duku, we concluded that they were similar genetic constitution and growth performance compared to the seedlings from the mother tree and all seedlings derived from embryo non-zygotic. This plant produced the offspring with the asexual system from the seeds by the apomictic reproduction.

ACKNOWLEDGEMENT

We would like to thanks Dr. Agus Susanto, Head of the Research Center of Tropical Fruit, Solok, West Sumatra, Indonesia for providing research facilities. Mr. Siddiq and Mr. Ahmad for sample collection. Parts of this study supported by Grant of Doctor Dissertation Research Ministry of Research, Technology and Higher Education, KP-Dikti: 066/SP2H/LT/ DRPM/IV/2017, and Cluster Research Grant of Professor Universitas Andalas, Indonesia No.33/UN.16.17/PP.HGB/LPPM 2017.

REFERENCES

Batygina TB, Vinogradova GY (2007). The phenomenon of polyembryony genetic heterogeneity of seeds. *Russ. J. Dev. Biol*. 38: 126-151.

Carman JG (1997). Asynchronous expression of duplicate genes in angiosperms may cause apomixes, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc.* 61: 51-94.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Irianto (2012). The phenophysiology of germination and growth of duku seedling (*Lansium domesticum* Corr.). *Bioplantae* 1:247-255. (In Indonesian language).

Kishore K (2014). http://www.research-gate.net./publication/131087846Polyem-bryony. Accessed 26th September 2017.

KoltunowAM, Hidaka T, Robinson SP (1996). Polyembryony in *Citrus* accumulation of seed storage proteins in seeds and in embryos cultured in vitro. *J. Plant Physiol*. 110: 599-609.

Kumar K, Mahanthi, Rani U (2013). Techniques to differentiate zygotic and nucellar seedlings in polyembryonic fruit crops. *Int. J. Agric. Environ. Biores.* 6: 344-350.

Mabberley DJ, Pannell JM, Sing AM (1995). Flora Malesiana. Series I–Spermatophyta. Flowering Plants. Volume 12 - Part I. Meliaceae. Foundation Flora Malesiana.

Mondal B, Pal A, Saha R (2014). Detection of zygotic embryos of *Citrus reticulata* by random amplified polymorphic DNA technique. *Int. J. Sci. Res.* 3: 2319-7064.

Murni P, Syamsuardi, Nurainas, Mansyah E, Chairul (2016). Genetic variability and out-crossing rate in the open pollinated duku 'Kumpe' (*Lansium parasiticum* (Osbeck) K.C. Sahni and Bennet.), a potential type of duku from Jambi, Indonesia. *Der Pharmacia Letter* 8: 185-191.

Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A (2009). Agro-forestree database: a tree reference and selection guide version 4.0 (*http://www.worldagroforestry.org/af/treedb/*).

Parab GV, Kirshnan S (2008). Assessment of genetic variation among populations of *Rhynchostylis retusa*, an epiphytic orchid from Goa, Indian using ISSR and RAPD markers. *Ind. J. Biotechnol*. 7: 313-319.

Ravishankar KV, Chandrashekara P, Sreedhara SA, Dinesh MR, Anand L, Saiprasad GVS (2004). Diverse genetic bases of Indian polyembryonic and monoembryonic Mango (*Mangifera indica* L) cultivars. *Curr. Sci.* 87: 870-871.

Salma I, Razali B (1987). The reproductive biology of Duku Langsat, *Lansium domesticum* Corr. (Meliaceae), in Peninsular Malaysia. *MARDI Res. Bull.* 15: 141-150.

Stefenova V, Bezo M, Labajova M, Senkova S (2014). Genetic analysis of three Amaranth species using ISSR markers. *Em. J. Food. Agric*. 26: 35-43.

Yeh FC, Yang RC, Boyle TJB, Ye ZH, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.

Yulita KS (2011). Genetic variations of *Lansium domesticum* Corr. accessions from Java, Sumatra, and Ceram based on random amplified polymorphic DNA fingerprints. *Biodiversitas* 12: 125-130.

Zukauskiene J, Paulauskas A, Varkuleviciene J, Marseliene R, Gliaudelyte V (2014). Genetic diversity of five different lily (*Lilium* L.) species in Lithuania revealed by ISSR markers. *Am. J. Plant Sci*. 5: 2741-2747.