Genetic Stability of Banana Plant Regenerated from Floral Axis Organogenesis Assessed by Newly Developed SSR Markers

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INTRODUCTION

Bananas have important roles not only in Indonesia but also world wide because of the economic aspect (FAO, 2003) and nutritional values (Hapsari & Lestari, 2016). In Indonesia, the socio-economic importance of banana has significantly increased across regions. The diverse utilization of the fresh and processed fruit is currently well recognized including traditional practices by local farmers that maintain diversity of local banana varieties (Hapsari, Kennedy, Lestari, Masrum, & Lestarini, 2017; Sato, 2009). However, currently there are some threats to the conservation and world banana production, such as commercialism that can lead to the extinction of local bananas, habitat destruction, and pest and disease risk (Panis, 2009). Therefore, in vitro method could be an alternative to regenerate banana plants for conservation purpose and mass production of banana planting materials.

Conservation method using in vitro culture is considered more efficient and effective than the conventional conservation in term of area, time, and cost (Govindaraju, Saravanay, Jayanthi, Nancy, & Indra Arulselv, 2012). However, regeneration technique should be well skilled on the in vitro application. In vitro culture in particular of organogenesis which is widely used in micropropagation and genetic transformation, showed a great progress in many plant species along with the use of growth promoting substances. In addition, an advance knowledge of morphological and physiological aspects is complemented the in vitro organogenesis and molecular mechanism (Izquierdo, González, & de la C Núñez, 2014). Organogenesis on banana has been progressively

ABSTRACT

Molecular marker is robust to precisely monitor the genetic stability of in vitro-banana plants. This study examined the genetic stability of 8 month-old banana plants of Soka variety derived from floral axis organogenesis using newly developed SSR markers. The results showed that the same qualitative and similar quantitative morphological characters of pseudostem, leaf and fruit were identified between mother plants and culture plants from floral axis regeneration. Both plants types were quite similar in number of tillers, brix percentage, fruit peel/mesocarp thickness and fruit length. Eleven out of 211 good quality of SSR loci showing high homology with important genes were selected for suitable PCR primers and produced unambiguous bands. The number of total bands was 323 for total SSR primers, in range of 20-60 per primer for total individual plants. Most culture plants showed identical with their mother plants, with very minor variation as reflected by genetic similarity coefficient range of 0.9-1.0. A high similar pattern on SSR to support morphological characters of mother plants and culture plants indicated a successful micropropagation using floral axis to encounter off-type clones. The floral axis organogenesis in this study is able to provide sufficient genetic materials of Soka for varietal registration and other applications.

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reported by using sucker (Roy et al., 2010; Shankar, Balaji, & Sekar, 2014), male flower (Sultan et al., 2011), and scalp (Elhory, Aziz, Rashid, & Yunus, 2009). To date, however, only few studies have reported the genetic stability of plants regenerated from in vitro organogenesis using floral axis of banana (Krikorian, Irizarry, Cronauer-Mitra, & Rivera, 1993; Martin, 2005), particularly in Indonesia (Lisnandar, Fajarudin, Effendi, & Roostika, 2015).

The most reliable method to assess the tissue culture-derived plants instability and variability are molecular markers. PCR-based molecular markers which include randomly amplify polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), simple sequence repeats (SSR), and start codon targeted (SCoT) are commonly used to observe clonal stability (Ateş Sönmezoğlu, Bozmoz, Yildirim, Kandemir, & Aydın, 2012; Procházková, Bousová, & Wilhelmová, 2011; Zietkiewicz, Rafalski, & Labuda, 1994). In particular, SSR marker is distributed throughout the plant genome, being species-specific, co-dominant and high polymorphic (Lakshmanan, Venkataramareddy, & Neelwarne, 2007).

Alternative choices to apply from low to high through put profiling, single to multiplex PCR, non- and fluorescent primers of SSR have been progressed (Culley et al., 2013; Ge, Cui, Jing, & Hong, 2014; James, Brown, Citroen, Rossetto, & Porter, 2011; Missiaggia & Grattapaglia, 2006; Schuelke, 2000). In regard to in vitro method, SSR profiles have been developed on different micropropagated clones, and the results showed true to type with the mother plants donor up to eight subcultures (Khan, Saed, & Kauser, 2011; Tiwari et al., 2013). Hundreds of SSR markers have been developed in banana and were predominantly in M. acuminata (Creste, Benatti, Orsi, Risterucci, & Figueira, 2006; Wang, Zheng, Huang, Liu, & Wu, 2010), a few were performed in M. balbisiana (Ravishankar et al., 2013). A total of 112 SSRs were identified in the 169 kb of plastid genome of two Musa species (M. acuminata and M. balbisiana) (Martin, Baurens, Cardi, Aury, & D’Hont, 2013). Twenty eight novel SSR markers from M. balbisiana were developed and used to access genetic variability of Thai bananas (Rotchanapreeda et al., 2016). SSR markers could help to establish the genetic fidelity of long-term micropropagated banana (Lakshmanan, Venkataramareddy, & Neelwarne, 2007). These progressive used SSR are valuable markers to assess molecular level of in vitro-regenerated banana plants worldwide. Notable, new SSR markers developed from the genome variant of banana originating from Indonesia would also be potential to assess genetic level of bananas from the country.

One of local highland banana in West Sumatera, called “Pisang Ayam”, is known as an edible banana and has long been cultivated in the rural community. Currently, this local banana is rarely grown and needs to be protected. The registration of this local banana to the authorized national organization in Indonesia (Center for Plant Variety Protection) by the local government allowed to rename it as “Soka”. An effective propagation approach for this important local banana variety is necessary to complement its morphological characters for the variety registration and other purposes. The aim of this present study was to examine the genetic stability of banana plants of Soka variety derived from floral axis explants through organogenesis regeneration based on the newly developed SSR markers.

MATERIAL AND METHODS

Plant Materials
A local banana variety of Soka originated from West Sumatera (Tanah Datar), Indonesia, was used in this study. The molecular analysis was conducted in the Laboratory of Molecular Biology, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development-Indonesian Agency for Agricultural Research and Development (IAARD), Bogor, West Java province. All banana plants have been grown and maintained under the management of Indonesian Tropical Fruit Research Institute (ITFRI)-IAARD, Solok, West Sumatera. This research had been carried out during 2015-2017.

In vitro Culture Method Regenerated from Floral Axis
Two male buds of the mother plant of Soka variety in field were collected and excised uniformly in size of 15 cm length. They were disinfected by dipping in detergent for 1 h followed by submerging in benomyl 10.4% and streptomycin sulphate 20% for next 1 h. They were sterilized with 70% ethanol, NaOCl 15.7% and NaOCl 10.5% for 5 min, respectively. Then, they were thoroughly washed with sterile distilled water for 5 min for three times.
The floral axis was excised transversal into 2 mm in size and subsequently cut a cross the central to get semi circle to be used as explant for each batch of tissue culture. The sterile explants were transferred to MS medium supplemented with 3 mg/l 6 benzylaminopurine (BAP) and 0.1% thidiazuron and 100 mg/l polyvinylpyrrolidone. Culture was under in vitro condition at room temperature (25 °C) with 16 h light, and intensity of 800-1000 lux. The explants culture was maintained for 1 month to turn green and subsequently for first subculture. For proliferation and elongation of shoot and root, the media contained 2.253 mg/l BAP, 0.175 mg/l indole acetic acid, and 100 mg/l ascorbic acid (Lisnandar, Fajarudin, Effendi, & Roostika, 2015). The successive subcultures were made based on the best results in the final morphological observation. Cycle subculture was carried out up to level two every 1 month interval.

Well grown shoots of 3-5 cm length were then subcultured on MS media containing of 0.5 % activated charcoal for root induction. Culture at second cycle was allowed for 3 weeks to get plantlets with healthy root, and shifted to acclimatization in the green house. About 50 banana plantlets were grown for 2 months in polybag containing soil mixture (sand, compost and soil with ratio of 1:1:2). These plants were maintained in shading net house for hardening, then moved to field and maintained well according to the recommended protocol of cultivation. Mother plants and in vitro culture plants have been maintained in the experimental field station.

DNA Extraction

Fresh leaves were harvested from five individual suckers of mother plant and 15 culture plants in the field for DNA extraction. About 0.5 g of the fresh leaves were grinded into a fine powder with mortar and pestle using liquid nitrogen. DNA extraction followed the cetyltrimethylammonium bromide (CTAB) method as described by Doyle & Doyle (1990). The DNA quantity and quality was determined using nanodrop2000 spectrophotometer (Thermo Fisher Scientific) at A_{260} and A_{280}. The DNA yield size was also estimated the concentration qualitatively on agarose gel 1% electrophoresis.

Development of SSR Primers

A number of SSRs were obtained by filtering of variants based on the alignment of the genomes of 12 local banana genotypes (data not shown). From the total sequences entry, 100% putative SSR primers retrieved with at least di-nucleotides were chosen. DNA sequences with good quality SSR motifs were used for designing SSR primer using BatchPrimer3 program (Rozen & Skaletsky, 2000).

PCR Amplification

Eleven out of total retrieved primers were selected and used to assess the genetic stability on 15 individuals of eight month-old plants regenerated from in vitro floral axis (called culture plants), and 5 suckers of mother plant as controls (called mother plants). The list of SSR primers with their sequences is presented in Table 1. SSR-PCR reactions were carried out in PCR T1 Thermocycler (Biometra, Germany). PCR reaction was made in a total volume of 10 µl contained 20 ng genomic DNA, 5 µl Kapa2G Fast ReadyMix (Kapa Biosystems, USA), 0.5 µl of each forward dan reverse (10 µM). The standard PCR was performed following the program of initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec, 55 °C for 1 min, and 72 °C for 1 min. A final extension was done at 60 °C for 15 min. The amplified DNA was analysed on 8 % polyacrylamide gel, then stained in ethidium bromide. DNA fragments were visualised under UV Transilluminator (Biorad, USA).

SSR Data Analysis

Amplicons of each primer on all individuals were scored and SSR allelic size was determined using Genetic Analyzer program. The size of each amplicon was estimated by comparing to 100 bp DNA ladder (Vivantis). Number of polymorphic bands produced by each primer was calculated. For dendrogram analysis, the scored data were converted to binary system and constructed using NTSYS. Nei’s genetic distance (Nei, 1973) was determined among the individual plant observed and used for grouping them by UPGMA (Unweighted Pair-Group Method with Arithmetic) clustering method (Rohlf, 2000).

Morphological Characterization

A total of 15 plants and 15 called mother plants were observed on their qualitative and quantitative morpho-agronomical characters until producing fruit. Fifteen qualitative morphological characters such as the shape of the stem cross section, pseudostem color, leaf blade shape, leaf blade color, midrib color, leaf sheath color, male bud shape, bract color, fruit shape, fruit apex, transverse
section of fruit, young fruit peel/epicarp color, ripen fruit peel/epicarp color, fruit pulp color, and fruit pulp taste were observed. While the quantitative morphological characters were observed including plant height, diameter of pseudostem at 50 cm from base, leaf blade length and width, male bud length and circumference, fruit length, fruit peel/mesocarp thickness, percentage of brix, and number of tillers. Morphological characterisation was carried out following the Descriptor for banana (IPGRI, 1996).

RESULTS AND DISCUSSION
Profile of Newly Developed SSR Markers

A nucleotide variation in the banana genome facilitates valuable markers either random throughout the genome or gene-specific sequences. SSR through in silico mining could be designed corresponding universal and/or genetic markers (Hippolyte et al., 2010). Genome sequence survey is an efficient way to develop SSR markers for wide application including characterisation with easy and low cost of data mining (Ravishankar et al., 2012). To facilitate the development of new SSR markers for banana, this research mapped the genome sequences of 12 banana genotypes performed by aligning with the reference genome (data not shown).

Hundreds of simple sequence repeats (SSRs) were identified and 211 loci were in good quality for further SSR primer development. All the flanking sequences of the 211 SSR loci allowed to design suitable PCR primers. The nucleotide sequences of the SSR which contained trinucleotides was the highest (38.9%), followed by dinucleotides (29.4%), tetranucleotides (17.5%), pentanucleotides (9.5%), and hexanucleotides (4.7%) across 12 chromosome of banana genome. The SSR motifs highly varied and the motifs of GA and AG were the highest frequency ranging from 5.2-5.7% in comparison to other repeat motifs. Primers were designed for the 211 loci and randomly 11 SSR located in chromosome 1 to 11 were selected for further synthesis of their oligonucleotides. The estimated PCR product size of the designed primers ranged from 300 to 400 bp. Further, based on the predicted genes using BLASTX algorithm, the flanking sequences containing SSR showed that nine were significant hits to *Musa* spp protein sequences with E-values near zero. These loci corresponded to RNA regulation of transcription (3 SSR), galacto-mannosidase (1 SSR), squamosal promoter binding like (1 SSR), transport function (2 SSR), cytochrome P450 (1 SSR) signalling receptor kinases (1 SSR), and no homology with any genes in database (2 SSR). The selected 11 SSR were designed their corresponding primers (Table 1).

Eleven SSR primers were then validated by genotyping of two genotypes i.e. Calcuta-4 which was re-sequenced its whole genome, and another parental genotype (*M. acuminata ssp. microcarpa*). This PCR amplification was to confirm these SSR primer design which was identified by in silico using bioinformatics. Seven lines derived from the crosses of Calcuta-4 and *M. acuminata ssp. microcarpa* were included in the primer confirmation. All the designed primers produced unambiguous amplicons (Fig. 1). These 11 SSR primers revealed polymorphism between the two parental lines and some lines possessed alleles belonging to one of the parents. Thus, these amplified DNA fragments produced by the SSR primers design proved their validity referring to in silico analysis.

Similar results of SSR marker development from genome sequences were investigated by several research groups (D’Hont et al., 2012; Hippolyte et al., 2010; Ravishankar et al., 2012; Venkataramana, Sampangi-Ramaiah, Ajitha, Khadke, & Chellam, 2015). SSR markers were considered suitable for assessing genetic stability and true to type of a number of micropropagated plants in many crop species, such as potato, *Dianthus giganteus subsp. banaticus*, *D. spiculifolius*, and *Pyrus pyraster*, etc. (Butiuc-keul, Farkas, & Cristea, 2016; Condello, Palombi, & Tonelli, 2008; Cristea, Crăciunăs, Marcu, Palada, & Butiuc-Keul, 2014; Jarda, Butiuc-Keul, Höhn, Pedryc, & Cristea, 2014; Tiwari et al., 2013). The SSR primers developed in this study could complement and enrich the existing SSR markers for banana species. Therefore, these polymorphic and scorable newly developed markers in this study, could be useful to broaden genetic studies in bananas including assessment of genetic stability of acclimated banana plants regenerated from floral axis-in vitro.

Assessment of Genetic Stability in Acclimated Banana Plants

Soka variety is a local banana originally from West Sumatera which is adaptive to the highland environment. In order to multiply this plant variety in short period and rapid, a micropropagation was performed to produce more clones, as an alternative method of the vegetative reproduction by sucker.
Table 1. List of newly developed SSR primers, their sequences and detailed information used in this study

| Primer name | Sequences | Chrom/position (bp) | Tm (°C) | GC (%) | Product size (bp) | Motif | Motif Length | SSR length | Predicted genes |
|-------------|-----------|---------------------|---------|--------|------------------|-------|--------------|------------|-----------------|
| MsSSR 1.1 | F = TCATCTGTAGACAGG AGATGC <br> R = TCTACTGTGATT TCGATGGAT | chr1:5984009 | 55.38 | 47.62 | 350 | GA | 2 | 20 | Galacto- and mannosidases, endoglucanase |
| MsSSR 2.1 | F = AGACAGTCACATTGCACATC <br> R = AGGATCCCAGTCTTAGATTCCG | chr2:13975828 | 55.83 | 42.86 | 351 | AG | 2 | 16 | Mitochondrial electron transport/ATP synthesis NADH:DH |
| MsSSR 3.1 | F = CCCACTCGCAATTAGAAT <br> R = CGGAGATGGATTAGGTATA | chr3:20984986 | 54.48 | 40 | 329 | GATT | 4 | 16 | No homology |
| MsSSR 4.1 | F = TTAAGAGGCGATGCAAATC <br> R = AAGCTCTACTGGTCTGTGCTA | chr4:3516258 | 55.18 | 33.33 | 316 | GGA | 3 | 12 | No homology |
| MsSSR 5.1 | F = AAGATGAACCTCAAGAAATTGATT <br> R = GGTCTCTCTGCTGAACTCAAT | chr5:9963330 | 54.8 | 33.33 | 350 | TA | 2 | 42 | RNA regulation of transcription. PHOR1 |
| MsSSR 6.1 | F = TCCTCCAGAAATTTGTAGA <br> R = GACTTCTCTGTGCTGTGATA | chr6:4064744 | 54.64 | 47.62 | 355 | CT | 2 | 28 | Development. squamosa promoter binding like (SPL) |
| MsSSR 7.1 | F = GAAAAAGTCCTCTTCAAAAGCT <br> R = ATTGGACTGCACTGCTGCG | chr7:2715453 | 55.41 | 42.86 | 353 | TACCG | 5 | 15 | Transport ABC transporters and multidrug resistance system |
| MaSSR 8.1 | F = CTCAGAAGATGGAGGGGATG <br> R = GTGACTCTGCTGTTGTTGAT | chr8:3827195 | 54.07 | 50 | 330 | GA | 2 | 14 | Misc. cytochrome P450 |
| MaSSR 9.1 | F = ATGTAGCAGGAAACAGACAAAGA <br> R = GACTCCTCATTATGGAACCG | chr9:17124975 | 55.1 | 33.33 | 357 | AGGA | 4 | 12 | Signalling. receptor kinases, wall associated kinase |
| MaSSR 10.1 | F = GAAGTCGAACCTGCAGTC <br> R = CAACCTCTGCTCTAAATGCTA | chr10:20676183 | 55.12 | 55.56 | 343 | TCT | 3 | 12 | RNA regulation of transcription. C2H2 zinc finger family |
| MaSSR 11.1 | F = ATGCGATCTCCCACGTAAT <br> R = AAGCGAGCTTCCAAGTAACTA | chr11:11676536 | 56.18 | 42.86 | 342 | GATC | 4 | 16 | RNA regulation of transcription. AP2/EREBP, APETALA2/ethylene-responsive element binding protein family |

Remarks: F = forward, R = reverse, Tm = melting temperature, SSR = simple sequence repeat

Fig. 1. Example of banding pattern of F1 progenies derived from the cross of Calcuta-4 and M. acuminata ssp. microcarpa as validation of newly designed SSR primers in this study. T1: Calcuta-4 (Parent 1), T2: M.acuminata ssp. microcarpa (Parent 2), F1a-F1g: lines 1-7.
Even though phenotype can be altered due to in vitro culture, such changes are often reversible (Khan, Saeed, & Kauser, 2011). However, optimal growth regulators, subculture cycles, explant source, and media composition were capable to induce the acclimated plants from in vitro to be true to type with the mother plants (Silvarolla, Mazzafara, & de Lima, 2000; Yu, Holland, McMullen, & Buckler, 2008).

Molecular markers are highly considerable used to monitor a nucleotide variation of plant species including banana. With the advantages of SSR which is abundant in genome, reproducible and simple, this molecular marker is reliable mean to analyse polymorphism (Khan, Saeed, & Kauser, 2011; Zerihun, Vashist, & Boora, 2009). In this study, the newly developed SSR markers demonstrated their potential application to evaluate the genetic stability of culture plants from floral axis organogenesis.

Based on the number and intensity of bands, 11 newly developed SSR markers showed banding pattern among individual plant. Only strong bands with eligible intensity were included for analysis. Most primers produced single band, but two primers (SSR5.1 and SSR8.1) had three bands. The number of total bands were 323 in range of 20-60 for total plants per primer with an average of 1.62 bands per primer per sample. About 243 bands were observed in the 15 individuals of culture plants that were higher than those of five individuals of mother plant as control (Table 2). The average bands resulted on individuals of mother plants and culture plants were relatively similar which were around 1.6, and it indicated their robustness. Some of banding pattern of SSR primers on the acclimated banana plants is presented in Fig. 2.

Dendrogram based on the banding pattern of the newly developed SSR markers confirmed that all individual plants of banana had very high genetic similarity (cut off of 0.9) as expected. There were very close relationship between mother plants and culture plants, with most of the genetic similarity coefficient of 1 (Table 3). A very low variation was found on the 8 month-old culture plants in the field (genetic similarity of 0.9 to 1.0), interestingly most of them were similar to the mother plants as demonstrated in the same cluster (Fig. 3). While three individuals of culture plant (no. 3, 9, and 11) were grouped in different clade but their genetic similarity coefficient was 1, suggesting their identical to each other. None of the scorable bands were found to be lost of original band of mother plant or gain of novel band in their regenerants, reflecting the high genetic stability.

The newly developed SSR markers showed their potential application for evaluation of the clonal genetic stability of Soka variety regenerated by floral axis organogenesis. In addition, the small number SSR markers out of thousands SSRs found in the genes of Indonesian local banana genotypes in this study could facilitate for other genetic studies. This results is relevant with the previous study by Venkataramana, Sampangi-Ramaiah, Ajitha, Khadke, & Chellam (2015) who explored approximately 4000 genic SSRs in M. balbisiana and M. acuminata which also proved the high genetic stability of in vitro regenerants. Floral axis is considered as a meristem tissue which actively divided and less contact to the ground (Resmi & Nair, 2011), therefore could eliminate the browning and budding propagation of banana, as demonstrated in this study.

In spite of the advantages of the in vitro propagation in particular organogenesis, a genetic instability occurred as a result of growth regulators. Additionally, repeated subculture activities trigger the emergence of different physiological responses between explant (Butiuc-keul, Farkas, & Cristea, 2016; Kour, Kour, Kaul, & Dhar, 2014). Moreover, high similar pattern on SSR and morphological characters of culture plants and mother plant, suggested a successful micropropagation using floral axis with optimal method to encounter off-to-type clones. The true to type clonal fidelity as an important prerequisite in the in vitro cultures, was prominently observed with high range of genetic similarity with their mother plant. Small variation of banana plants (2%) in this study also indicated its permissible to deal with the commercial practical micropopagation outfits (François-Xavier, Sandoval, Marie, & Auboiron, 1993). Therefore, this study proved that all newly developed SSR markers were applicable to examine Soka clonal genetic stability and could be applied to other banana varieties.

**Morphological Characterization of In vitro-Regenerated Plants in the Field**

Banana plant commonly has a maximal vegetative development and start flowering at around eight months after planting. The characterization of its morphology at given developmental stage would allow plant to be mature which expectedly represent genetic variability with less bias.
Fig. 2. SSR banding profiles of acclimated banana plants derived from organogenesis of floral axis in comparison with the suckers of mother plant of Soka variety visualized on polyacrylamide gel 8 % using primer MaSSR 5.1 (A) and MaSSR 10.1 (B). M:DNA ladder, lane 1-5:suckers, 6-20:individual plants derived from organogenesis of floral axis.

Table 2. Scoring profile of monomorphic bands produced by SSR markers in Soka variety

| Regenerated plants | Individuals | SSR1.1 | SSR2.1 | SSR3.1 | SSR5.1 | SSR6.1 | SSR7.1 | SSR8.1 | SSR9.1 | SSR10.1 | SSR11.1 | Total of bands |
|--------------------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------------|
| Suckers (control)  | 1           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 2           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 3           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 4           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 5           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
| Total of bands for suckers |               | 10     | 5      | 5      | 15     | 10     | 5      | 15     | 5      | 5      | 5      | 80             |
| Average no. of bands for sucker |             | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 1.6            |
| Floral axis clones |             |        |        |        |        |        |        |        |        |        |        |                 |
|                    | 1           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 2           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 3           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 4           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 5           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 6           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 7           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 8           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 9           | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 10          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 11          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 12          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 13          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 14          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
|                    | 15          | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1      | 1      | 1      | 16             |
| Total of bands for floral axis |               | 30     | 15     | 15     | 45     | 30     | 15     | 45     | 18     | 15     | 15     | 243            |
| Average number of bands for floral axis clones |             | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1.2     | 1      | 1      | 1.6            |
| Total of bands per primer |               | 40     | 20     | 20     | 60     | 40     | 20     | 60     | 23     | 20     | 20     | 323            |
| Average number of bands per primer |               | 2      | 1      | 1      | 3      | 2      | 1      | 3      | 1.15    | 1      | 1      | 1.62           |

Remarks: red circle= polymorphic band
Table 3. Genetic similarity matrix of individual banana of mother plants (sucker) and culture plants (floral axis organogenesis)

| Plants | S1   | S2   | S3   | S4   | S5   | Fa1  | Fa2  | Fa3  | Fa4  | Fa5  | Fa6  | Fa7  | Fa8  | Fa9  | Fa10 | Fa11 | Fa12 | Fa13 | Fa14 |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S1     | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S2     | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S3     | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S4     | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S5     | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Fa1    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Fa2    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |
| Fa3    | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 1.00 |      |      |      |      |      |      |      |      |      |      |
| Fa4    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 |      |      |      |      |      |      |      |      |      |
| Fa5    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 |      |      |      |      |      |      |      |      |
| Fa6    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |      |
| Fa7    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |      |
| Fa8    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |      |      |      |      |      |
| Fa9    | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 1.00 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 1.00 |      |      |      |      |
| Fa10   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 |      |
| Fa11   | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 1.00 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 1.00 | 0.90 | 1.00 |
| Fa12   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 |
| Fa13   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 |
| Fa14   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 |
| Fa15   | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 | 0.90 | 1.00 |

Remarks: S:sucker, Fa:floral axis clone

Fig. 3. Dendrogram of acclimated plants from *in vitro*-floral axis and the mother plant-suckers based on coefficient similarities generated by the UPGMA cluster analysis using the NTSYS-program.
In general, similar morphological performance of pseudostem, leaf and fruit were identified in all individuals of both mother plants and culture plants at the same developmental phase (Fig. 4, Table 4). The pseudostem shape and color were rounded and green-red. The color of leaf blade and midrib was green, with flat shape and both rounded of leaf blade, while leaf sheath color was light green. The male bud was ovoid with red purple color of bract. The peel of immature fruit or epicarp color was green and became bright yellow when ripen, with yellow pulp and the shape of slightly curved. The pulp has similar sweet/good taste. Both fruit peel/mesocarp thickness and fruit length ranged 11.0-13.5 cm, and its brix was 28-29%. Both mother plants and culture plants also had similar male bud size and number of tillers (2-4). Nonetheless, the culture plants tend to be higher in plant height and pseudostem diameter. In addition, leaf blade of culture plants tended to be smaller than that of mother plants. Notable, the most identical culture plants with mother plants demonstrated that this in vitro approach in this study did not change their genetic level, as reflected by their similar morphologies. Moreover, minor morphological variations recorded in some clones indicated an individual variability to respond environment but not a genetic inheritance.

**Table 4.** Morphological characters of acclimated plant clones regenerated from *in vitro* floral axis compared to the mother plants from suckers cultivated in Tanah Datar, West Sumatera

| Characters                                | Suckers (mother plants) | Culture plants |
|-------------------------------------------|-------------------------|---------------|
| **Qualitative**                           |                         |               |
| The shape of the pseudostem cross section| Rounded                 | Rounded       |
| Pseudostem color                          | Green-red               | Green-red     |
| Leaf blade shape                          | Flat and both rounded   | Flat and both rounded |
| Leaf blade color                          | Green                   | Green         |
| Midrib color                              | Green                   | Green         |
| Leaf sheath color                         | Light green             | Light green   |
| Male bud shape                            | Ovoid                   | Ovoid         |
| Bract color                               | Red purple              | Red purple    |
| Fruit shape                               | Slightly curved         | Slightly curved |
| Fruit apex                                | Pointed                 | Pointed       |
| Transverse section of fruit               | Rounded                 | Rounded       |
| Young fruit peel/epicarp color            | Green                   | Green         |
| Ripen fruit peel/epicarp color            | Bright yellow           | Bright yellow |
| Fruit pulp color                          | Yellow                  | Yellow        |
| Fruit pulp taste                          | Sweet/good taste        | Sweet/good taste |
| **Quantitative**                          |                         |               |
| Plant height (cm)                         | 273 – 318               | 310-358       |
| Pseudostem diameter (cm), at 50 cm from base | 20 – 27               | 21 – 29       |
| Leaf blade size: length (cm)              | 300 – 318               | 250 – 260     |
| width (cm)                                | 80.0 – 85.0             | 72.0 – 85.0   |
| Male bud size: length (cm)                | 18.0 - 25               | 18.0 - 22     |
| circumference (cm)                        | 29 - 33                 | 30 - 34       |
| Fruit length (cm)                         | 11.0 – 13.5             | 11.5 – 13.5   |
| Fruit peel/mesocarp thickness (mm)        | 2.0 – 3.0               | 2.0 – 3.0     |
| Brix (%)                                  | 28-29                   | 28-29         |
| Number of tillers                         | 2 – 4                   | 2 – 4         |
Fig. 4. Banana plant of Soka variety, where A-D= culture plant from floral axis organogenesis clone, E-F= suckers from mother plant. A) Two month- old culture plants grown in sand medium in polybag, B) Three month-old culture plant from transplanted ones in the polybag which were well cultivated in the field, C) Inflorescence, D) Male bud, E) A bunch of immature fruit produced by plant regenerated by in vitro, F) Mother plant with immature fruit (8 old-month age).
Stable clones were produced via regeneration of floral axis organogenesis as demonstrated in this study, to complement vegetative propagation (Vincent & Anushma, 2018). Being as dessert banana, Soka variety is most likely to have AAA genome or probably AAB genome, indicating this variety is closer genetically to its ancestral parent, M. acuminata than M. balbisiana. Compared to other organs, to use floral axis as in vitro culture materials is proven to be successful and rapid, in which to proliferate shoot and root in such banana species with genome type which is usually resulted low proliferation. This result is also in accordance to a previous study which reported a high rate of nodule and shoot on organogenesis of floral axis of AAB and ABB genomes (Lisnandar, Fajarudin, Effendi, & Roostika, 2015). Furthermore, high number of clones could be generated by adding ascorbic acid to reduce browning (Kariyana & Nisyawati, 2013), as revealed in this study. Second cycle of subculture in this study allowed to obtain high similarity of acclimated plant of Soka variety, suggesting that minimal subculture cycle is preferable. As previously reported that high subculture level, the genotype explant, and prolonged light exposure could depict tremendous genetic variation (Poerba, Imelda, & Martanti, 2012; Ray, Dutta, Saha, Das, & Roy, 2006). Phytohormones and repeated in vitro subculture and duration may increase mutation and lose the regeneration potential (Muhammad, Rashid, Hussain, & Naqvi, 2007), consequently it is recommended to be eliminated as demonstrated in this study. In addition to the genetic stability of this Soka variety, it was clear that the morphological characteristics of both mother plants and culture plants, were specific to its genomic group (Hapsari & Lestari, 2016), suggesting that the morphological characters revealed the same between mother plant and culture plants (fruit peel/mesocarp thickness: 2.0-3.0 mm, brix percentage: 28-29%, number of tillers: 2-4), and quite similar on fruit length. The minimum variation among them were detected on plant height, diameter of pseudostem, leaf blade size and male bud size.

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