Morphological and Simple Sequence Analysis of Tangerine (*Citrus nobilis* L.) From Three Regencies in North Sumatra

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Abstract. Tangerine or Mandarin orange (*Citrus nobilis* L) is a well known citrus in the world and becomes a fruit commodity in Indonesia. Various types of local tangerine have been produced with different name depends on the village and Regency where the citrus are grown. However, the information about the genetic variation of the citrus are limited. The research is aimed to study the genetic similarity of the local tangerines by using morphological and simple sequence analysis. The research was carried out by collecting citrus samples from eight villages that are spread in three Regencies at North Sumatera. The morphological characteristic of the citrus are documented, and the DNA are analyze by using simple sequence repeat (SSR) for their genetic variation. The results have revealed that different tangerines (local named as Brastepu, Maga, Sipirok) are having similar morphological characters.

The protein in the DNA are containing 48 bands (100 bp-300 bp), consisted of 30 polymorphic bands and 18 monomorphic bands, and have compared to four DNA primers. Analysis of the genetic diversity by using NTsys software found that they are clustered on 0.74 similarity coefficient value and the local citrus are devided into 3 groups. The lowest genetic distance on the Sibanggor Tonga with Baringin Siumuran was 0.63 (63%), meanwhile the highest distance was 1.0 (100%) on Huta Namale with Huta Lombang, Aek Kambiri, and Aek Horsik.

1. Introduction

Citrus can be categorized as the most cultivated fruits in the world, and they are consisted of various species with different taste. The most well known citrus is sweet orange (*Citrus sinensis* L. Osbeck) that are widely consumed fruits in the world. Citrus was originally planted in South East Asia and spread throughout the world, including Indonesia [1]. The name for citrus can be vary based on the growth location, fruit shape, quality, embryo types, inflorescence, tree growth and habit, and adaptability. However, the taxonomy has been based mainly on morphological data and geographical data. Tangerine or Mandarin orange (*Citrus nobilis* L) is the most widely planted in Indonesia [2]. Tangerine citrus are grown well and become a potential commodity from North Sumatra [3]. The citrus are named differently from one place to another depend on the places where the tangerine are grown, such as Brastepu from Kabupaten Karo, Maga from Kabupaten Mandailing Natal, and Sipirok from Kabupaten Tapanuli Selatan [4]. The genetic of the citrus become a germplasm asset in Indonesia, and therefore, the diversity of the citrus is needed to be investigated to have a clear understanding on the variety of local citrus in Indonesia.

Several studies have been conducted to investigate the diversity of the citrus, including the molecular characterization [5] and genetic variations as reported in the references [6,7]. The investigation starting from its basic chromosome number, the genome size, and the variety of the DNA
The reference genetic map of sweet orange has also been reported [9,10]. The genetic maps by using molecular markers have been developed and are used as a tool to select important traits in the breeding program associated with their pathogen resistance and fruit quality of the target plants in the propagation of good seedling [11]. Various types of molecular markers have been applied to elucidate the genetic architecture of quantitative traits for agronomic interest [12]. The method by using Simple Sequence Repeat (SSR) markers has widely used for genetic diversity analysis that are characterized from the level of polymorphism, codominant, multi-linear, and is well used for the determination of genetic diversity [13,14]. The RT-PCR method for DNA expression pattern has also been reported [15]. There are many type of sweet orange are grown in North Sumatera which are known differently by local citrus. However, there is no study has been conducted on the genetic characteristic of the local citrus that are assigned to be very important to trace the origin of sweet orange in North Sumatera. Therefore, the analysis of citrus genetic has to be conducted to obtain a well documented genetic of the local citrus. The study is aimed to identify the genetic similarity of the citrus tangerine (Citrus nobilis L) from three regencies in North Sumatra by using of morphological and simple sequence analysis. The work is intended to give the information on the genetic diversity of tangerines in the province.

2. Method

The research are consisted of sample citrus collection, morphology identification, isolation and quantification of the DNA, and PCR-SSR analysis. The collection of Tangerine or Mandarin orange (Citrus nobilis L) leaves are carried out from three Regencies in North Sumatera Indonesia, namely Kabupaten Karo (local name is Brastepu), Kabupaten Mandailing Natal (local name is Maga), and Kabupaten Tapanuli Selatan (local name is Sipirok). Identification of the leaves are carried out by visual and descriptive characterization in the field based on the leaves performances (shape, tips, colour, and the surface), the stem (the shape and colour), and the fruits and seeds [3] (Nurwahyuni & Sinaga, 2014). Laboratory experiments were carried out in the Laboratory Genetic, Department Biology, Faculty of mathematics and Natural Science (FMIPA) Universitas Sumatera Utara, Medan, Indonesia. Extraction of the DNA and the PCR-SSR analysis are performed followed the procedures explained earlier [2,16]. The quality of the DNA was assessed from its electrophoresis agar gel prepared in TAE buffer (Tris free base, Glacial Acetic Acid, and 0.5 M EDTA at pH 8.0), and the DNA was quantified by using nanophotometric at λ 260 nm. The PCR-SSR analysis was conducted by using of 12.5 µL 2X Dream Taq Green PCR Master Mix (Thermo Scientific), 1 µL forward primer, 1 µL reverse primer, 2 µL of the DNA template, and 8.5 µL water free nucleate [17]. The band of the SSR gel profile was scored manually with consideration of the presence and the absence of the band [18]. The genetic variation of the citrus was analyzed by using numerical taxonomy and multivariate analysis system (NTSys) that were presented as dendrogram with un-weighted pair-group with arithme average (UPGMA) method [19]. The experimental details are available in the references [4].

3. Results and Discussions

The results and discussion for this study are consisted of 5 subsections, namely Description of Data Collection, Morphological Characteristic of Citrus, Isolation and Analysis of the Citrus DNA, Analysis of DNA Genome, and Genetic Variation of Tangerine Citrus. The results will be presented in the sections and discussed accordingly.

3.1. Description of Data Collection

The samples are obtained from various places (Villages) spread around three Regencies in North Sumatera, and the description of the samples are listed in Table 1. The samples are consisted of healthy citrus which are named as Tangerine Brastepu (TB), Tangerine Maga (TM), and Tangerine Sipirok (TS), and the condition of the citrus plants are vary from young, mature and old trees where most of them are in healthy conditions and still in high productivity, and only one citrus tree was unhealthy and unproductive. The samples are met the criteria to be qualified samples for this study.
Table 1. The descriptions of citrus samples, the location (Village and Regency) where were the citrus samples are taken, and the condition of the citrus tree samples.

| No. | Name of Local Citrus (Tangerine) | The Village and Regency Where the Trees are Grown | General condition of citrus sample |
|-----|---------------------------------|-----------------------------------------------|-----------------------------------|
| 1   | Tangerine Brastepu (TB)         | Berastagi, Kabupaten Karo                      | Old, healthy, ±10 years old, and productive |
| 2   | Tangerine Maga 1 (TM1)          | Sibanggor Tonga, Kabupaten Mandailing Natal    | Mature, healthy, ±5 years old, and productive |
| 3   | Tangerine Maga 2 (TM2)          | Huta Namale, Kabupaten Mandailing Natal        | Old, unhealthy, ±9 years old, and productive |
| 4   | Tangerine Maga 3 (TM3)          | Huta Lombang, Kabupaten Mandailing Natal       | Old, healthy, ±8 years old, and productive |
| 5   | Tangerine Sipirok 1 (TS1)       | Banjar Tikus, Kabupaten Tapanuli Selatan       | Old, healthy, ±9 years old, and unproductive |
| 6   | Tangerine Sipirok 2 (TS2)       | Baringin Siiumuran, Kabupaten Tapanuli Selatan | Mature, healthy, ±6 years old, and productive |
| 7   | Tangerine Sipirok 3 (TS3)       | Aek Kambiri, Kabupaten Tapanuli Selatan        | Young, healthy, ±4 years old, and productive |
| 8   | Tangerine Sipirok 4 (TS4)       | Aek Horsik, Kabupaten Tapanuli Selatan         | Mature, healthy, ±6 years old, and productive |

3.2. Morphological Characteristic of Citrus

The morphological of citrus samples are analysed based on the phenotype characteristic which are seen from the leaves, fruits, and stems, and the results are summarised in Table 2. The colour of the leaves are mostly identical, while the colour and the shape of ripe fruits are different from one citrus to another tangerines. The stems of mature trees are also different from one citrus to another tangerine trees, the height of mature trees can be reach up to 8 meters height where some of them has produce fruits at 2-4 metres tall. Most of the tangerines are having thorns in the stem and branches, those are the tangerine that are produced via generative method, while some local citrus are without thorns, those are the tangerines that are propagated through grafting method [21,22]. The trees are mostly having many branches and the location of the branches are scattered with shady canopy. Those morphological characteristic are used to distinguish the citrus. However, another factors such as the environmental condition, soil nutrients, and the climate conditions have to be considered to influence the grove of the citrus trees in the area where they grown [22]. Morphological characteristic of the tangerines are tends to be similar that are collected from different districts. These morphological data become preliminary identification that the tangerines have similarity in the genetic.

Table 2. Morphological characteristic of Tangerine (Citrus nobilis l.) from three regencies in North Sumatra, Indonesia.
Local Citrus | Morphological characteristic (Phenotive) | Leaves | Fruits | Stems |
---|---|---|---|---|
Tangerine Maga (TM) | Margin type *repandus*, *ovate*, *acutus*, without wing, shiny leaf surface, dark green in upper side and light green in the bottom. | Round flat shape, the tip with notched edge, short neckline, ripe fruit yellow to orange with shiny skin. | Mature tree 6-8 m with thorns, and containing many small branches. |
Tangerine Sipirok (TS) | Margin type *repandus*, *lanceolat* (spear), *acutus* type tips, without wing, shiny leaf surface, dark green in upper side and light green in the bottom, plat leaf position | Round flat shape, ripe fruit is yellow to orange with porous skin, the end of the fruit is flat until notched. | Mature tree 3-8 m with thorns, and containing many small branches. |
Tangerine Braste (TB) | Margin type *repandus*, *lanceolat* (spear), *acutus* type tips, without wing, shiny leaf surface, green in upper side and light green in the bottom, plat leaf position. | Round flat shape, ripe fruit is orange to light red with shiny skin. | Mature tree 2-8 m, m without thorns, memiliki duri, and containing many small branches. |

3.3. Isolation and Analysis of the Citrus DNA

There are eight citrus samples are collected to represent three type of Tangerine and their DNA have been extracted and the electrophoresis analysis and PCR amplification have been carried out. The DNA profiles showed that the extracted samples are free from contaminations and the DNA conditions are suited for analysis for the samples size of the DNA-genomes are all $\geq 100$ bp [23]. The purity and the concentration of citrus DNA-genomes are presented in Table 3. Within the samples, the highest concentration of the DNA was observed in the TS1 (835 ng/µL) and the lowest concentration was found in TM1 (264 ng/µL). The purity of tangerines’ DNA was observed ranges from 1.775 - 1.986, in which the best was obtained in TM1 (1.986). With these conditions, all DNA samples are suited for further analysis for variation of the genetic diversity [24].

Table 3. The concentration profiles of the DNA genome of various local citrus in North Sumatera

| No. | Local Citrus | The purity of the DNA | Concentration of the DNA (ng/µL) |
|---|---|---|---|
| 1 | Tangerine Braste (TB) | 1.886 | 368 |
| 2 | Tangerine Maga 1 (TM1) | 1.986 | 264 |
| 3 | Tangerine Maga 2 (TM2) | 1.792 | 345 |
| 4 | Tangerine Maga 3 (TM3) | 1.786 | 710 |
| 5 | Tangerine Sipirok 1 (TS1) | 1.785 | 835 |
| 6 | Tangerine Sipirok 2 (TS2) | 1.775 | 750 |
| 7 | Tangerine Sipirok 3 (TS3) | 1.808 | 368 |
| 8 | Tangerine Sipirok 4 (TS4) | 1.832 | 340 |

3.4. Analysis of DNA Genomes
The amplification and analysis of the DNA-genome have been carried out for citrus samples and the band profiles are analysed by using of SSR primer standards. The band profiles have showed that most of the samples are containing protein that similar to the primer with different size (Figure 1). The electrophoregraph revealed that the samples are containing the band profile similar to the DNA ladder, and the DNA profile with four primers are summarised in Table 4. The band profiles that are compared to TAA15 primer have showed that the DNA ladder consisted of three protein in different size (300 bp, 250 bp and 100 bp), while compared to TAA27 primer the DNA profiles containing of three protein (200 bp, 150 bp and 100 bp), while the profile that are compared with TAA41 primer are found contained three proteins (200 bp, 150 bp, and 100 bp), and there are two bands are obtained by using CAC23 (200 bp and 100 bp).

**Table 4.** Band profiles of the DNA genome extracted from various local citrus in North Sumatera, and the proteins are compared to four type of citrus primers standards.

| No. | Type of primer | Total band profile | Locus polymorphic | Locus monomorphic |
|-----|----------------|-------------------|------------------|------------------|
| 1   | TAA15          | 11                | 5                | 6                |
| 2   | TAA27          | 15                | 14               | 1                |
| 3   | TAA41          | 13                | 8                | 5                |
| 4   | CAC23          | 10                | 4                | 6                |
|     | Total          | 49                | 31               | 18               |

**Figure 1.** The SSR DNA profiles of three local Tangerine citrus that are compared to the citrus markers (M) 100 bp, by using primers of: (a) TAA15, (b) TAA27, (c) TAA41, and (d) CAC23.
The results showed that three local citrus provided from 8 locations are having 49 band profiles (Table 4), where the primers are containing of 10-15 bands. The there are 15 bands are observed when using TAA27 primer, followed by lower bands profile successively TAA41 primer (13 bands), TAA15 primer (11 bands) and CAC23 primer (10 bands). The polymorphic band profiles was obtained the most with TAA27 primer (14 bands), and the lowest band profiles was obtained by using CAC23 primer (4 bands). The monomorph band profiles have been obtained by all four primers, where each of the TAA15 and CAC23 primers are having 6 bands, and TAA41 primers contained 5 bands, while TAA27 primers was only 1 band. The variation in the bands profiles by using different primers could be caused by the purity and the concentration of the DNA in the samples [24,25].

### 3.5. Genetic Variation of Tangerin Citrus

Variation of the genetic for local citrus in North Sumatera has been studied, that was from citrus samples that are collected from different villages. Similarity in the genetics were analyzed for Tangerin Sipirok and Tangerin Maga as simulated in genetic matrix in Table 5 and Table 6. The details on the genetic similarity for Tangerin Sipirok was obtained between TS3 and TS4 (100%) where both are identical, while genetic similarity between TS1 with TS4 and TS3 was obtained 72% (see the results in Table 5).

**Table 5. Similarity analysis for citrus genetic of Tangerin Sipirok based on the band profiles of the DNA genome compared to four citrus primers standard.**

|       | TS1  | TS2  | TS3  | TS4  |
|-------|------|------|------|------|
| TS1   | 100% |      |      |      |
| TS2   | 80%  | 100% |      |      |
| TS3   | 72%  | 89%  | 100% |      |
| TS4   | 72%  | 89%  | 100% | 100% |

The same procedures to observe the genetic variation for Tangerin Maga has also been used as presented in Table 6. The amplification has produce 20 of DNA profiles. The samples which are similar in the genetic between TM2 and TM3 (100%), where the similarity in the genetic between TM1 with TM2 and TM3 (67%). Although all samples are provided from different villages in one region (Kabupaten Mandailing Natal), but the genetics are far from one to another. The reason for the variation of the genetic for local citrus might be due to the breeding procedures that have been conducted to obtain citrus seedling. The farmer are tend to apply grafting technique to propagate local citrus seedling with hoping that the generating plants with have high productivity similar genetic profile from its mother plants [2]. However, the cross-breeding between the plants has possibly occurred during the plant developments that deviated from its original genetic profile.

**Table 6. Similarity analysis for citrus genetic of Tangerin Maga based on the band profiles of the DNA genome compared to four citrus primers standard**

|       | TM1  | TM2  | TM3  |
|-------|------|------|------|
| TM1   | 100% |      |      |
| TM2   | 67%  | 100% |      |
| TM3   | 67%  | 100% | 100% |
Analysis of the data by using NTSys software showed that the similarity coefficient of 0.63 was obtained from genetic grouping of eight citrus which is means that the tangerines are tend to be similar to its parents. There are three groups are obtained from citrus collections of tangerines which cluster randomly, they are (1) Group 1 consisted of the Brastepu (TB) and Sibanggor Tonga collections (TM1) which are originating from Kabupaten Karo (Berastagi) and Kabupaten Mandailing Natal, (2) Group 2 consists of 5 collections filled by 2 districts, namely Kabupaten Mandailing Natal consisting of Huta Namale (TM2) and Huta Lombang (TM3), and Kabupaten Tapanuli Tengah consisting of Aek Kambiri (TS3), Aek Horsik (TS4), and Baringin Siumuran collections. (TS2), and (3) Group 3 was only occupied by the collection of Banjar Tikus (TS1) originating from Kabupaten Tapanuli Tengah.

The highest genetic similarity in the tangerine populations are found in Huta Namale, Huta Lombang and Sipirok. There are individuals are found at Aek kambiri, Aek Horsik with similarity coefficient values 100% (1.0) and they are situated in the same group. Therefore, the members in the population of Maga and Sipirok tangerines carry the same genetic traits and characteristics. With this reason, the tangerines are having close relationships and the descended are coming from one ancestor [26]. The dendogram to show the genetic relationship of the citrus is presented in Figure 2.

Low genetic similarity for Maga Tangerine was obtained from Huta Namale and Huta Lombang, where for Sipirok Tangerine was obtained from Aek Kambiri, Aek Horsik, Baringin Siumuran, Banjar Tikus with a similarity coefficient value of 74% (0.74), and another citrus such as Brastepu Tangerine and Maga Tangerine from Sibanggor Tonga are having similarity in the genetic with a coefficient value of 84% (0.84). It is concluded from the results that the character of those tangerines have changes from their ancestors [27]. The cultivation system with grafting method have promoted the change in the genetic since the farmers tends to select the buds from a good tree and pasted it in to selected mother plants that are resistant to pests or diseases, may become major influence on the emergence of new diversity. For this reason, the changes in the character or the nature of the tangerines affected the low coefficient value of the genetic diversity of the local citrus in North Sumatera.

4. Conclusions
The research has concluded that the genetic similarity of the local tangerines of Brastepu, Maga and Sipirok are observed even though they are grown in different villages in North Sumatra. The molecular analysis of eight tangerines samples has been compared to show that the similarity in the leaves, fruits and stems are exists. The DNA's of local citrus have successfully extracted and are run
for electrophoresis profiles followed by RSS analysis. The DNA of the tangerines have been compare with four primers where the citrus contain of 48 band profiles (100 bp-300 bp), 30 of them are polymorphic bands and 18 monomorphic bands. The highest polymorphic bands were obtained at TAA41 primers with 14 DNA bands and the lowest as many as 4 DNA bands on CAC23 primer. The dendogram revealed that the genetic relationship of the tangerines are positive with high similarity coefficient value.

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References
[1] Ollitrault, P., Terol, J., Chen, C., Federici, C.T., Lotfy, S., Hippolyte, I., Ollitrault, F., Bérard, A., Chauveau, A., Cuenca, J., Costantino, G., Kacar, Y., Mu, L., Garcia-Lor, A., Froelicher, Y., Aleza, P., Boland, A., Billot, C., Navarro, L., Luro, F., Roose, M.L., Gmitter, F.G., Talon, M., and Brunel, D., 2012. BMC Genomics 13: 593. http://www.biomedcentral.com/1471-2164/13/593.
[2] Nurwahyuni, I., and Sinaga, R., 2018. Pakistan Journal of Botany 50(2): 667-678.
[3] Nurwahyuni, I., & Sinaga, R., 2014. Int J Pharm Bio Sci 5(4): (B) 863 - 873.
[4] Pangaribuan, N.C., 2018. Analisis Keanekaragaman Genetik Jeruk Keprok (Citrus nobilis) Sumatera Utara Menggunakan Marka Simple Sequence Repeat (SSR), Skripsi, Departemen Biologi, FMIPA USU.
[5] Ruiz, M., Pensabene-Bellavia, G., Quiñones, A., García-Lor, A., Morillon, R., Ollitrault, P., Primo-Millo, E., Navarro, L., and Aleza, P., 2018. Frontiers in Plant Science 9: Article 901 (22 pages), doi: 10.3389/fpls.2018.00901.
[6] Nurwahyuni, I., Napitupulu, J.A., Rosmayati, and Harahap, F., 2015. Journal of Agricultural Science 7(4): 30-39.
[7] Donmez, D., Simsek, O., Izgu, T., Kacar, Y.A., and Mendi, Y.Y., 2013. The Scientific World Journal, Article ID 491207, (8 pages) http://dx.doi.org/10.1155/2013/49120.
[8] Shimizu, T., Kitajima, A., Nonaka, K., Yoshioka, T., Goto, S., Toyoda, A., Fujiyama, A., Mochizuki, T., Nagasaki, H., Kaminuma, E., and Nakamura, Y., 2016. PLoS ONE 11(11): e0166969. doi:10.1371/journal.pone.0166969.
[9] Wang, Y., Zhou, L., Li, D., Dai, L., Lawton-Rauh, A., Srimani, P.K., Duan, Y., and Luo, F., 2015. PLoS ONE 10(3): e0121893. doi:10.1371/journal.pone.0121893.
[10] Ollitrault, P., Terol, J., Chen, C., Federici, C.T., Lotfy, S., Hippolyte, I., Ollitrault, F., Bérard, A., Chauveau, A., Cuenca, J., Costantino, G., Kacar, Y., Mu, L., Garcia-Lor, A., Froelicher, Y., Aleza, P., Boland, A., Billot, C., Navarro, L., Luro, F., Roose, M.L., Gmitter, F.G., Talon, M., and Brunel, D., 2012. BMC Genomics, 13: 593 http://www.biomedcentral.com/1471-2164/13/593.
[11] Curtolo, M., Cristofani-Yaly, M., Gazaffi, R., Takita, M.A., Figueira, A., and Machado, M.A., 2017. BMC Genomics 18: 289 (16 pages), DOI 10.1186/s12864-017-3629-2.
[12] Omura, M., and Shimada, T., 2016. Breeding Science 66: 3–17. doi:10.1270/jsbbs.66.3.
[13] Shimizu, T., Tanizawa, Y., Mochizuki, T., Nagasaki, H., Yoshioka, T., Toyoda, A., Fujiyama, A., Kaminuma, E., and Nakamura, Y., 2017. Frontiers in Genetics 8: Article 180, (19 pages). doi: 10.3389/fgene.2017.00180.
[14] Jannati, M., Fotouhi, R., Abad, P.A., and Saleh, Z., 2009. Journal of Horticulture and Forestry. 1(7): 120-125.
[15] Zamharir, M.G., Hamzeh, K., Alizadeh, A., and Kachoei, S., 2014. *International Journal of Agriculture and Crop Sciences* **7**(15): 1509-1513.

[16] Garcia-Lor, A., Curk, F., Snoussi-Trifa, H., Morillon, R., Ancillo, G., Luro, F., Navarro, L., and Ollitrault, P., 2013. *Annals of Botany* **111**: 1–19, doi:10.1093/aob/mcs227.

[17] Golein, B., Talaie, A., Zamani, Z., Ebadi, A., and Behjatnia, A., 2005. *International Journal of Agriculture and Biology* **7**(2): 167-170.

[18] Liu, C., Jiang, D., Cheng, Y., Deng, X., Chen, F., Fang, L., Ma, Z., and Xu, J., 2013. *PLOS ONE* **8**(3): e58411 (9 pages).

[19] Xie, R., Li, Y., He, S., Zheng, Y., Yi, S., Lv, Q., and Deng, L., 2014. *PLoS ONE* **9**(12): e113971. doi:10. 1371/journal.pone.0113971.

[20] Martasari, C., and Supriyanto, A., 2005. *Prosiding Seminar Nasional Jeruk Tropika Indonesia*. pp. 36-53.

[21] Nurwahyuni, I., Marpaung, H.N., and Rahayu, S., 2017. *Int. J. Pharm. Bio. Sci.* **8**(4) (B): 30-39.

[22] Hardiyanto, Mujiarto, E., and Sulasmi, S.E., 2007. *Jurnal Hotr* **17**(3): 203-216.

[23] Sankar, G.T., Gopi, V., Deepa, B., and Gopal, K., 2014. *Int J.Curr. Microbial. App. Sci.* **3**(4): 75-84.

[24] Langga, F.I., Restu, M., and Kuswinanti, T., 2012. *Jurnal Sains dan Teknologi*. **12**(3): 265-276.

[25] Wang, Y., Atta, S., Wang, X., Yang, F., Zhou, C., and Cao, M., 2018. *PLoS ONE* 13(6): e0198022. https://doi.org/10.1371/journal.pone.0198022.

[26] Hidayat, T., and Pancoro, A., 2008. *Jurnal Agrobiogen*. **4**(1): 35-40.

[27] Zheng, B., Wu, X., Ge, X., Deng, X., Grosser, J.W., and Guo, W., 2012. *PLOS ONE* **7**(8): e43758 (13 pages).