Genetic purity and population structure of potato variety Granola L.

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Abstract. The phenotypic performances have formed the basis for genetic purity evaluation. However, the phenotypic based genetic purity assessment are time-consuming, expensive, and not stable due to a great environmental effect on traits expression. While, the genotypic based genetic purity evaluation of a variety offers an efficient, more stable, and precise result than those phenotypic method due to no environmental factors involved. The objectives of this study were to determine genetic purity and population structure of Granola L. potato variety derived from twelve collection sources using microsatellite markers. This study consisted of following steps, such as cultivation of Granola L. potato varieties in the field, DNA isolation, PCR analysis, amplified DNA fragment analysis. The results showed that Granola L. variety derived from twelve sources had high level of genetic purity which indicated by genetic similarity value of 0.96 observed in present study. Based on population structure analysis, Granola L. variety might have consisted of two sub-populations of which one sub-population was more dominant than other sub-population. Therefore, genetic purification activities for Granola L. variety are still needed to increase genetic purity and to reduce phenotypic differences in future.

1. Introduction
Granola L. is a potato variety that was originally come from the selection of German potato cv. Granola. This variety was released by the Indonesian government in 1993. Based on the variety description, Granola L. variety showed high yielding (26.5 tons/ha), good tuber quality and suitable for table potatoes. In addition, this variety Granola L. resistant to Potato Virus A (PVA), Potato Virus Y (PVY), and Potato Leaf Roll Virus (PLRV), but moderately susceptible to bacterial wilt and leaf rot diseases [1]. The market demand for Granola L. variety is very high in Indonesia, making it the preferred variety which is widely grown by Indonesian farmers. However, as a variety that has been released for decades, some farmers reported that the performance of Granola L. variety has been phenotypically changed.

These variations might be caused by two possible factors either internal or external factors. The
internal factors, changes in phenotypic variation could be due to repeated sub culturing activities of the respective variety that result in soma clonal variations. While, the external factors causing such phenomenon might be due to the use of local or unregistered imported seeds for certain variety. Type of these Granola seeds is mostly an essential derivative of Granola L. or imported Granola and the cultivation of this Granola seed would usually provide. Phenotypic variations may be indicated by the decrease in genetic quality of the seeds. Therefore, it is importance to evaluate the genetic quality or genetic purity of certain variety including potato Granola L. in breeding program.

At present, the genetic quality of a variety is commonly examined through morphological or phenotypic observations. However, the phenotypic examined is less stable due to phenotypic appearance can be influenced by the environmental factor. Tanksley and Mc Couch [2] reported that the assessment of genetic quality of varieties through the application of DNA markers resulted in high accuracy due to no environmental factors influenced the genotyping analysis. Recently, a lot of molecular markers have been developed and their sequences can be easily accessed by user. Of these, microsatellite or simple sequence repeats (SSRs) marker is a type of DNA marker that is widely used. This SSR consists repetition 1–6 base pairs of DNA with high variation [3]. In present study, SSR markers were applied in ascertaining the genetic purity of Granola L. variety. It is expected that the use of SSR markers would be accurate and reliable tool for testing the genetic purity of Granola L. compared to that based on conventional method phenotypic analysis.

To date, The Indonesia Center for Agricultural Genome, known as PGPI has successfully performed whole genome sequence of five Indonesian potato varieties [4]. Based on these potato genome sequences, a set of microsatellite markers distributed on 24 potato chromosomes has also been developed [5]. Of these, a total of 24 SSR markers developed from the PGPI potato database has been validated by genotyping such new developed SSR markers on 14 potato genotypes [6]. In present study, such SSR markers developed by the PGPI were applied to test both the genetic purity and the study of the structure of long-established potato populations. The objective of the present study was to assess the genetic purity and determine the population structure of Granola L variety using microsatellite markers. Results from this research is expected to produce information on the genetic purity of the Granola L population structure, and can be utilized by IVEGRI or seed producer to produce Granola L. potato seeds with high genetic quality.

2. Materials and Methods

Plant genetic materials used in present study consisted of twelve Granola L. potato genotypes collected in IVEGRI’s seed production unit, seed bank of ICABIOGRAD, and seed producer which supervised by IVEGRI in Lembang, Pangalengan, Garut, Banjarnegara, and Pasuruan. The research was conducted at the Laboratory of Molecular Biology and in the Research Station of IVEGRI in Lembang, West Java Province started from January to December 2019.

2.1. Cultivation Practices

Potato tubers of 35-50 mm in size were planted in some number of plots following the standard cultural practices. Each plot was 5m long and 0.5m wide with 20 plants per plot and the distance between plots was 80–30cm. Organic and mineral fertilizer were applied to the field experiment. Horse manure (30 t/ha) was applied one week before planting, while mineral fertilizers were properly applied at the rate of 1 t/ha. Plant pests and diseases were intensively controlled during the study.

2.2. DNA isolation

DNA isolation of twelve Granola L. potato variety were carried out based on CTAB DNA extraction method with minor modification [6]. Genomic DNA was isolated from healthy and young potato leaves (10 plants per genotype that randomly selected). Isolated DNA was then stored in a deep-freezer at -20°C. The quality (purity) and quantity (concentration) of isolated DNA were determined using a spectrophotometer. The performance of each isolated DNA sample was also observed by electrophoresis and visualized using the gel documentation tool (Biorad).
2.3. PCR (Polymerase Chain Reaction) analysis

PCR of twelve Granola L. potato variety was conducted using 18 SSR primers from PGPI [4,5] (Table 1). Amplification was performed in a thermal cycle (Biorad) with a 25 μL of total volume. PCR reaction was consisted of 1X GoTaq green master mix (Promega), 0.25 μM of each primer (forward and reverse), 20 ng of genomic DNA, and 9.5 μL RNase-free water.

| Markers | Forward | Reverse | Annealing (°C) |
|---------|---------|---------|---------------|
| StSSR1.1 | CAGTGGGAGCAATAATAATCA | TGAGCTAGACTTGGTCTCAAA | 53 |
| StSSR2.1 | AACTTTGGATCTACCTCGTTC | ACAAAAAGAATGACATTGACGTA | 52 |
| StSSR3.1 | GTGACCTCTGATGTTGCTAAC | TCCTGGAGTGCTCTATTATG | 52 |
| StSSR4.1 | CAGTTATGAAAAATCTGGTCAA | ACCAAGTGACACATGCAATAA | 52 |
| StSSR5.1 | AATTGAGTGACCATTGAGAAA | GACTAACCACATACCAACATCA | 50 |
| StSSR5.2 | CCGGATGTTACATTGGAGAGATG | TTGGTTTTATGAAGATGGAG | 51 |
| StSSR6.1 | CAAGGAAGGTGGAGACCTGAAG | AGTTAAAGCTGATGATCAAA | 51 |
| StSSR7.1 | CAACATTATTATCTTTGCACCAAC | TTTGTTTTTATGAAGATGGAG | 48 |
| StSSR8.1 | GACGATTGTTGATCCGTGTTGAT | TAAAAGGACGACACAAATCA | 50 |
| StSSR8.2 | AAAATGATGACAGTGGAGATG | GACTAACCACATACCAACATCA | 51 |
| StSSR9.1 | TTGGTGGATCTAATTTGCTAGTT | ATGTGAGTTGAGGAGATGGAG | 51 |
| StSSR9.2 | CAAAGTTACATGAAGTGTGTC | ATGTGAGTTGAGGAGATGGAG | 51 |
| StSSR10.1 | TTGGTGGATCTAATTTGCTAGTT | TGAACCTAATCAACACCTTGA | 50 |
| StSSR10.2 | GAAACCGTGCTTGATTTGAACTG | GCACCTATCTAAACAGCAATA | 50 |
| StSSR11.1 | TTCAGAAACCTGTAATCTTCCAAA | AATAAAGCTTGCTGTGTATGC | 52 |
| StSSR11.2 | AGCAAAATATGAGACAGTT | GCAACACTGAGTTGCTTC | 50 |
| StSSR12.1 | TTTGTGTATGAGGAAGCTCAC | CCTGTACATCAATCTGGACT | 49 |
| StSSR12.2 | TAGATTGGAGGACAGAAT | CCATAGCGAGGACCTGAT | 52 |

The optimum PCR condition was initial denaturation at 95 °C, 30 seconds for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, primer annealing (Table 1), and primer extension at 72 °C. The final 5 minutes extension was then set up at 72 °C. PCR products were separated using agarose gel electrophoresis in 0.5 TAE buffer. A total of 5 μL PCR products mixed with 1 μL loading dye, and 1 μL sybr green along with 100 bp DNA ladder were loaded onto 4% agarose gel that has been placed in a mupid-e Xu electrophoresis system (Advance co. ltd.) for 25 minutes, 100 volt. Gel was visualized using the gel documentation tool (Biorad).

2.4. Fragment analysis

Fragment analysis uses a Gen-Analyzer program was used to read the molecular weight of visualized bands. The molecular weight data of each PCR product becomes raw data for the dendrogram construction which will explain the genetic purity of Granola L. The dendrogram constructed based on algorithm paired group (UPGMA) and the Bray-Curtis similarity index using Past Program [7]. The genetic purity was also confirmed by GenAIEx software [8]. The number of sub-populations was performed using Structure [9] and the most K value was processed with Structure Harvesters [10]. The final output of these program was in the form of a color chart that reflects the number of sub-populations.

3. Results and Discussion

Genetic diversity of the Granola L. derived from twelve sources genotyped by 18 SSR markers was presented as dendrogram that clearly produced two main clusters. Clusters I consisted of 11 Granola L varieties and one Granola L remained in second cluster (Cluster II). Based on dendrogram (Figure 1). The twelve Granola L varieties was clearly separated at the genetic similarity coefficient of 0.96,
reflected the genetic variation value of 0.4. This result revealed that low genetic variability among the twelve Granola L. varieties used in our study.

![Dendrogram level of purity / genetic similarity of potato Granola L varieties from twelve sources using 18 SSR primers and Sample of SSR profiles using two primers.](image)

**Figure 1.** (a) Dendrogram level of purity / genetic similarity of potato Granola L varieties from twelve sources using 18 SSR primers; (b) Sample of SSR profiles using two primers.

There were several statements about the genetic variation based on the genetic similarity from the genotyping analysis. Zhang et al. [11] and Salimi et al. [12] stated that the genetic similarity value of 0.71 still demonstrated a high polymorphic rate, implying rich genetic diversity and strong environmental adaptability in the species/variety. Basaki et al. [13] had genotyped many accessions of Iranian *R. persica* using AFLP markers, and also observed that some populations had less than 0.5 genetic similarity to each other reflected low genetic variation. While Tinche et al. [14] state that genetic similarity which was reached 0.56 in UPGMA analysis showed a genetic composition that did not differ much. Based on our clustering analysis, the Granola L. varieties derived from twelve source showed high level of genetic purity which indicated by genetic similarity value of 0.96.

In addition to clustering analysis, confirmation of genetic purity of the twelve Granola L. varieties evaluated in present was also carried out using population structure analysis. In previous studies, population structure is widely used to study genetic diversity of both plants and animals [15-18]. The use of the population structure approach in the genetic diversity study of potato genotyped has been reported [11,19,20]. In present study, the population structure analysis on Granola L. derived from twelve sources is expected to help us in elucidating the number of sub populations in the certain potatoes of the Granola L. variety. The results showed that the population of Granola L. from twelve sources was divided into two sub-populations (Figure 2). Of these, one sub-population is more dominant than another sub-population. These results were in accordance with those by clustering analysis which described in a dendrogram (Figure 1). In other words, the twelve Granola L. varieties showed the level purity of 96% suggested a 4% of genetic differences.
Figure 2. (a) Bayesian model-based estimation of structure (K=2) for the twelve Granola L. varieties. Groups are separated into different color. Numbers on the y-axis showed coefficient of membership/assignment, while the numbers on the x-axis indicated each Granola L. variety; (b) The structure harvesters result

The two sub-populations obtained in the structure analysis reflected that these varieties have a narrow population structure and low genetic diversity in the population [11,19,20]. In this study, the twelve Granola L. varieties using 18 SSR markers were clustered into two sub-populations, which means that those potato varieties showed a narrow population diversity. Up to date, based on consumer’s preference of Granola L. variety is still greatly preferred in comparison to the other potato varieties and even more new released superior varieties. Therefore, the improvement of potato varieties focused by introgression of one or two important traits such as diseases resistance to develop new superior varieties with Granola L. as genetic background. However, Juyo et al. [20] reported that continuous manipulation activities in one cultivar can cause a narrower genetic basis.

Overall, the results of this study indicated that the Granola L. variety has high genetic purity but on contrary, several morphological characters of those Granola L. derived from twelve sources showed differences. This contradictory result might be caused by the effect of environmental on the morphological characters as widely known that phenotypes can be influenced by genetics, environment, and their interactions factors. Another factor, the seed management might be the reason why the morphology change that occurred in Granola potato farmer field. However, the evaluation of Granola L varieties at molecular level is very important in monitoring the occurrence of genetic change in Granola L derived from different sources to ensure their genetic purity. The low number of SSR and sample use on this study might be contributed to the high genetic similarity, the next study will include more sample. Moreover, the information of genetic purity would be beneficial to help breeders, especially those working on potato breeding program in IVEGRI or seed producer to produce Granola L. potato seeds with high genetic quality.

The assessment of genetic purity at molecular level is more accurate and stable compared to that at morphological level that resulted in unstable phenotypes appearance. Some studies reported that phenotypic diversity is usually greater than the genotypic diversity of a character [18,21,22]. To minimize phenotypic variation, it is recommended to tighten the selection of the off type in the seed production process of Granola L. Completing the Granola L. description in accordance with UPOV standards and carrying-out periodic purification process are efforts that can be made to improve genetic purity and phenotypic uniformity.

4. Conclusion
Based on the assessment of genetic purity at molecular level, Granola L. variety derived from twelve sources had high level of genetic purity and narrow population structure.

Acknowledgments
The authors are very much thankful to IVEGRI for financial support (DIPA-Balitsa-2019: 1804.208.053.D.3), laboratory technical support (by D M Sakti and N Nuraini), and data analysed support (by P Ependi).

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Author’s contributions
All authors contributed equally to this work. RK designed the experiments, analysed data, and wrote the manuscript. K and AKK helped in material collection. R and NG reviewed and edited the manuscript. CH designed the experiments and supervised the project.