BioEksakta: Jurnal Ilmiah Biologi Unsoed
Volume 2, Nomor 1 (2020): 1 - 6
E-ISSN: 2714-8564

Molecular Phylogeny of Sunflower Cultivars of Teddy Bear, Skyscraper, Lemon Queen and Common Sunflower Using RAPD Markers

Gabriella A Tanudharma*, Nurtjahjo Dwi Sasongko, Alice Yuniaty

Fakultas Biologi, Universitas Jenderal Soedirman
Jalan dr. Suparno 63 Purwokerto 53122
*email : gabriellegchen@gmail.com

Article History:

Received : 27/08/2019
Accepted : 20/01/2020

Abstract
Helianthus or sunflower is a genus of plant comprising about 70 species. Common sunflower and other members of Helianthae are cultivated in temperate regions and some tropical regions as food crops for humans, cattle, poultry, and as ornamental plants. The common sunflower is valuable with respect of economic and ornamental point of view. There are many cultivars of sunflower including teddy bear, skyscraper, and lemon queen. Variation among these cultivars has been studied using molecular techniques and the result were used to develop the phylogeny among them. Random Amplified Polymorphic DNA (RAPD) is one of molecular techniques that were used for this purpose. The purpose of this study was to contruct the phylogeny of three sunflower cultivars and common sunflower based on RAPD markers. The RAPD primers used in this study were OPA-2, OPA-9, OPA-13, OPB-2, OPB-4, OPB5, OPB-7, and OPB-11. Data analysis based on molecular data showed that genetic relationship among Lemon Queen, Skyscraper, Teddy Bear and Common sunflower based on RAPD markers shows that the cultivars studied are grouped into three main groups, namely: Group I Lemon Queen and Skyscraper, Group II Teddy Bear, and Group III Common sunflower; the closest kinship is shown between Lemon Queen and Skyscraper.
Keywords: *sunflower; genetic diversity; primer; RAPD; phylogeny.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) the main cash crop and is a member of Asteraceae which is the largest family of flowering plants, with over 25,000 species. Experts divide this family into about 15 subunits called tribes (Smith, 2014). Sunflower belongs to genus Helianthus which is one of the largest of Asteraceae family with about 2,000 species. Helianteaes special features are having heads with both central disk and outer ray flowers (these often yellow in color, some red or purple), an imbricate involucre and paleate receptacle, and carbonized cypselas with a pappus of awns or scales (Vear, 2016).

In tropical countries the sunflower can grow well in both season (Hockett & Knowles, 2018). The sunflower is grown manly for its oil-seeds and the seeds. This crop has scored fourth position between oilseed crops. It even topped over palm oil, brassica and soybean (Badouin *et al*., 2017) with the oil-seed is considered premium oil and is good for health. Seeds of this crop contain important fatty acids with 49% oil.

Current sunflower's genetic study includes sunflower genetic diversity, sunflower DNA sequencing and phylogeny has significantly increased the progress and approaches of new technologies and methods for the crops development and how to efficiently utilize them. The information obtained from the study increases the chance to produce new types of sunflower which are more resistant to pests and weeds, and also have higher production rate of seeds and oil-seed (Tomlekova *et al*., 2014).

The importance of studying genetic diversity in sunflower is to know the uniqueness or special traits of every type of sunflower to be able to efficiently utilize them. DNA sequencing is important for marking specific genes which responsible for certain trait of sunflower such as oil-seed production. Phylogeny, especially molecular phylogeny of sunflower has been continuously developed to find which group of sunflowers have better producibility on certain utility. For example, gigantic sunflowers have the best resistancy to weeds, while dwarf sunflowers have the best oil production (Hockett & Knowles, 2018).

In this study, randomly amplified polymorphic DNA (RAPD) were used because this analysis is commonly used for studying taxonomy of various organisms (Suresha, 2017). An RAPD assay is rapid and easy to perform and also requires only small amount of DNA. Earlier, inbred lines were discriminated only on the basis of morphological characters. With the development of various PCR based marker technologies including RAPD, variations among the lines can be observed at the DNA level. The purpose of this study was to construct the phylogeny of three sunflower cultivars and common sunflower based on RAPD markers

MATERIAL AND METHOD

Materials used in this were sunflower of Lemon Queen, Skyscraper, Teddy bear sunflower cultivars and common sunflower. The study consisted of three sequential steps namely DNA extraction, quality and quantity testing of the isolated genomic DNAs, and RAPD marker amplification. Genomic DNAs was isolated using GeneAid plant DNA kit. The quality of genomic DNA was tested using electrophoresis with 1% agarose gel. Quantity of the isolated genomic DNA was tested using UV spectrophotometer at λ 260 to 280 nm absorbance. This study was conducted for six weeks, from May to June 2019 in Genetic and Molecular Laboratory, the Faculty of Biology, Jenderal Soedirman University.

PCR was done in a total reaction volume of 12,25 µL, consisting of 6,25 µL mytaq PCR mix, 10 ng DNA template, 1 µL primer of 10 µM, and 5 mL nuclease-freewater. DNA amplification was performed using PCR machine peqlab primus250. Amplification started with pre-denaturation at 950C for three minutes, followed by 35 cycles od denaturation at 950C for 15 seconds, annealing at 310C for 15 seconds, elongation at 720C for 10 seconds and was ended with one cycle of complete extention at 720C for 5 minutes. The PCR products were visualized using 1,5% agarose gel electrophoresis technique using 1X TBE buffer. Visualization was performed using UV transluminator.

The analysis of RAPD bands profile was done by descriptive method. DNA banding pattern that emerges from each individual were used to determine the polymorphism among samples. A band was said to be polymorphic when the occurrence is less than 95%. The PCR products was scored separately based on it presence or absence of the bands using binary code. Value of 1 is given if a band is present and 0 if the band is absent.

Table 1. PCR Primers

Primer	Sequence (5'-3')	Source
OPA-2	TGCCGAGCTG	(Griffin & Annete, 1994)
OPA-9	TGCCGAGCTG	(Griffin & Annete, 1994)
OPA-13	CAGCACCCAC	(Griffin & Annete, 1994)
OPB-2	TGATCCCTGG	(Griffin & Annete, 1994)
OPB-4	GGACTGGAGT	(Griffin & Annete, 1994)
OPB-5	TGCGCCCTTC	(Griffin & Annete, 1994)
OPB-7	GGTGACGCAG	(Griffin & Annete, 1994)
OPB-11	GTAGACCCGT	(Griffin & Annete, 1994)

RESULT AND DISCUSSION

DNA Isolation

The result of genomic DNA isolation is presented in Figure 1. All genomic DNA are seen to be in good quality with minimum smear.

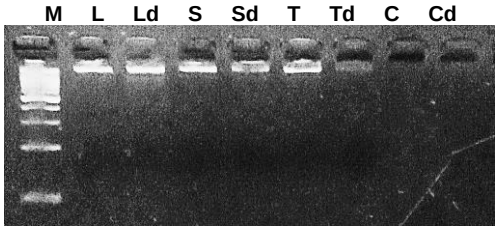

Figure 1. Genomic DNAs of three sunflower cultivars and Common sunflower **(**M= marker, L= Lemon Queen sunflower cultivar, S= Sky Scraper sunflower cultivar, T= Teddy bear sunflower cultivar, C= Common sunflower, all samples are duplicated (d))

The concentration of the genomic DNAs range from 10,0 to 63,5ng/µl. This means that are the genomic DNAs are in sufficient amount to be used as templates RAPD marker amplification. Therefore, all samples except Teddy Bear duplo are diluted to obtain DNA concentration of 10,0 ng/µl as DNA template for PCR-RAPD.

Tabel 2. DNA concentration and absorbance ratio of three sunflower cultivars and Comoon sunflower

Sample	DNA concentration (ng/µl)	A260/280 absorbance ratio
Lemon Queen	38.5	1.878
Lemon Queen duplo	37.5	1.875
Skyscraper	24.5	1.885
Skyscraper duplo	15.0	2.000
Teddy Bear	44.5	1.816
Teddy Bear duplo	10.0	1.800
Common	20.5	2.050
Common duplo	63.5	1.954

PCR-RAPD Amplification

Lemon Queen, Skyscraper, Teddy Bear sunflower cultivars and Common sunflowers' RAPD profiles are shown in Figure 2 with polymorphic bands pointed with arrows. The figure shows that OPA-2 primer is capable to amplify 7 loci consist of 22 DNA bands ranged between 180-950 base pairs. The DNA band will be scored if it appears on the sample although it does not appear on the duplo sample. Two loci are polymorphic with percentage of 33.33%.

OPA-9 primer is capable to amplify 9 loci consist of 22 DNA bands ranged between 380-1500 base pairs. Seven loci are polymorphic. The polymorphism percentage is 77,78%. OPA-13 primer is capable to amplify 10 loci consist of 16 DNA bands ranged between 280-1000 base pairs. Nine loci are polymorphic. The polymorphism percentage is 90%.

OPB-2 primer is capable to amplify 4 loci consist of 15 DNA bands ranged between 180-290 base pairs. One locus is polymorphic. The polymorphism percentage is 25%. OPB-4 primer is capable to amplify 5 loci consist of 11 DNA bands ranged between 490-1300 base pairs. Four loci are polymorphic. The polymorphism percentage is 80% A monomorphic band is very thick probably because the DNA fragment has many copy numbers or DNA fragments that have not been separated because the agarose gel can not separated DNA fragments different only few base pairs.

OPB-5 primer is capable to amplify 8 loci consist of 28 DNA bands ranged between 250-1000 base pairs. Four loci are polymorphic. The polymorphism percentage is 50%. OPB-7 primer is capable to amplify 7 loci consist of 23 DNA bands ranged between 350-950 base pairs. Two loci are polymorphic. The polymorphism percentage is 28,57%. OPB-11 primer is capable to amplify 8 loci consist of 20 DNA bands ranged between 150-520 base pairs. Five loci are polymorphic with the polymorphism percentage is 62,5%.

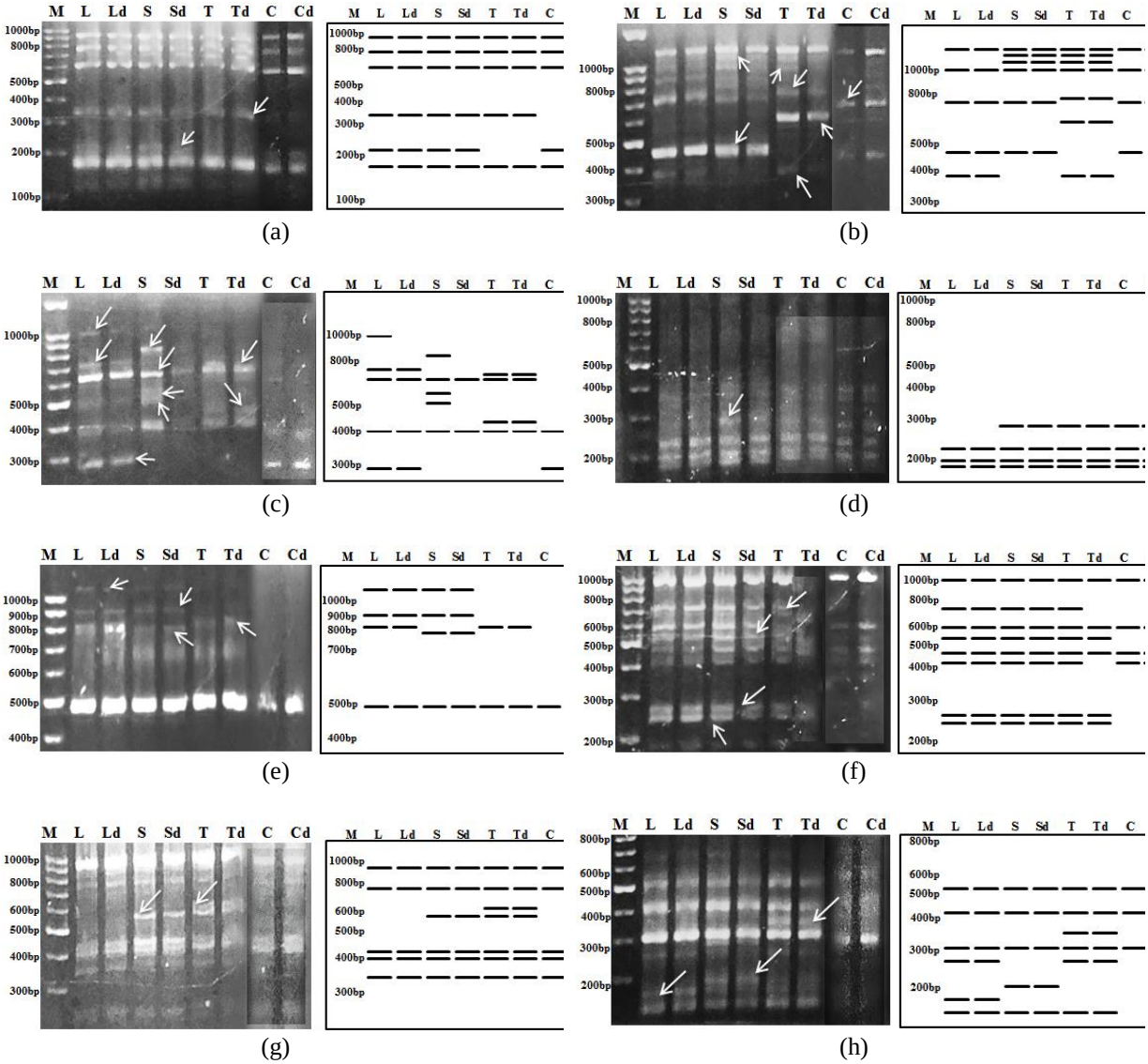

Figure 2. Lemon Queen, Skyscraper, Teddy Bear sunflower cultivars and Common sunflower using RAPD primer OPA-2 (a), OPA-9 (b), OPA-13 (c), OPB-2 (d), OPB-4 (e), OPB-5 (f), OPB-7 (g), and OPB-11 (h)

Notes : M = marker, L = Lemon Queen sunflower cultivar, S = Sky Scraper sunflower cultivar, T = Teddy bear sunflower cultivar, C = Common, all samples are duplicated (d).

The RAPD results show varied results for each primer. This variation appear because RAPD primers have random sequences that do not have any specific genome sequence. They will attach to complementary sequences of the RAPD markers (Darvishzadeh, 2016). The primers with the highest number of DNA bands is OPB-5 (Table 3), in contrast primer OPB-4 amplifies the least DNA bands (Table 3).

The difference in the number of bands depends on the ability of each primer to recognize and amplify DNA fragments. In addition, the difference in amplification results can also be caused by annealing temperature in the PCR process is less ideal because each RAPD primer has an ideal temperature requirement while in this research the same annealing temperature is used for all primers (Griffin & Annete, 1994).

Overall RAPD profiles show that the common sunflower sample has the most different profile compared to the other three cultivars. Tomlekova *et al*. (2014) stated that many bands that appear in the Lemon Queen, Skyscraper and Teddy Bear sunflower samples tend to not appear in the common sunflower which grow in Russia. In addition, common sunflower is rarely crossed because this type is considered to have mediocre characters except for its resistance ability.

Tabel 3. Numbers of RAPD bands amplified from 8 primers

Primer	Number of polymorphic bands	Number of monomorphic Bands	Total bands	Polymorphism percentage
OPA-2	2	4	6	33,33%
OPA-9	7	2	9	77,78%
OPA-13	9	1	10	90,00%
OPB-2	1	3	4	25,00%
OPB-4	4	1	5	80,00%
OPB-5	4	4	8	50,00%
OPB-7	2	5	7	28,57%
OPB-11	5	3	8	62,50%
Total	34 59,65%	23 40,35%	57 100%	

Sunflower Phylogenetic Relationship

Analysis of molecular phylogeny was carried out to obtain information about molecular character of each individual. The molecular character can be used as a basic information about the potential of each tested cultivar (Ellur *et al*., 2015). The phenogram of the sunflower cluster analysis using the MEGA 7.0 UPGMA program on the matrix of the genetic distance of Lemon Queen, Skyscraper, Teddy Bear and Common Sunflower is presented in Figure 3.

.

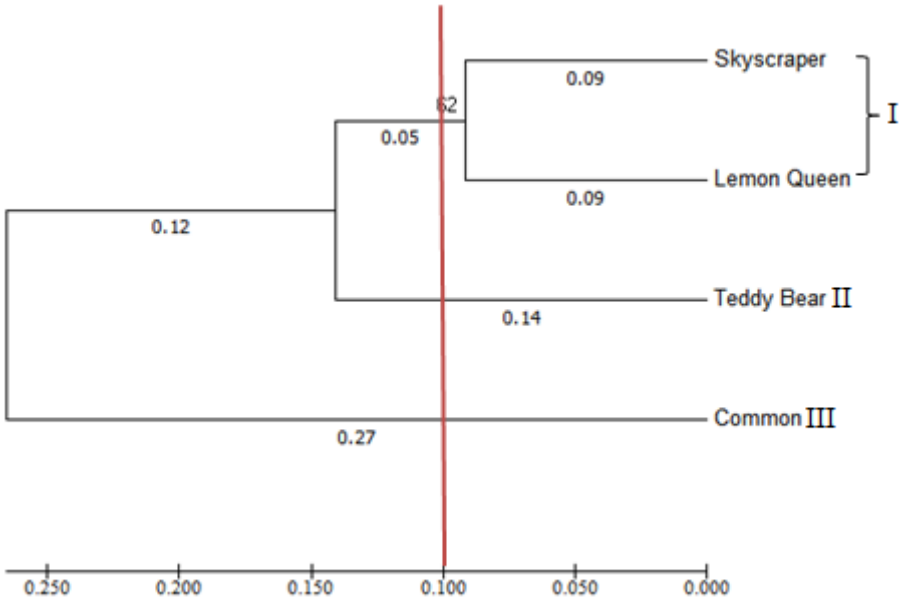

Figure 3. Phenogram of Sunflower Molecular Phylogenetic Relationship

Based on the phenogram, each cultivar can be distinguished from one another. If a hypothetical line is drawn that cuts phenogram at a coefficient of 0.100, three groups will be formed, namely group I which consists of Lemon Queen and Skyscraper sunflowers, group II consists of Teddy Bear sunflower, and group III consists of Common sunflower.

P-distance value between Skyscraper and Lemon Queen is the lowest (0.183) which means that the genetic distance is the closest compared to other cultivars. Interestingly, both cultivars share similarities in several morphological characteristics such as : flower head size, flower head type, ray floret shape (Mandel *et al*., 2014), flowering period, flowering phase (Nascimento *et al*., 2016), hypocotyl pigmentation, bract shape, bract length, leaf serration, leaf hairiness, leaf wings expression, leaf angle of lowest lateral veins, leaf height of the tip compared to insertion of petiole (Masvodza, 2015), stem attitude, stem hairiness, stem diameter (Jannatdoust *et al*., 2016), sowing to emergence phase, emergence to maturity of 5 pairs of leaves phase (Beard & Geng, 1982), achene' stripe color (Sudrik *et al*., 2014), seed type and seed oil content (Premnath *et al*., 2016).

Teddy bear belongs to group II because it has different RAPD profile (6 out of 8 primers) shows that this cultivar is different from the other 2 cultivars. These molecular data are supported by morphological classification like dwarfism. However, it has some similarities with group I in the following characters : leaf serration, shape of leaf cross section, shape of leaf distal part (Masvodza, 2015) and branching type (Hockett & Knowles, 2018), These similarities maybe caused teddy to be closer to group I than Common sunflower.

Common sunflower belongs to group III shows that it is significantly different from the other three cultivars. This information is supported by the p-distance value between Common Sunflower and Lemon Queen sunflower (0.670) as the highest p-distance value. It shows that the genetic distance of common sunflower to theother three cultivars is the furthest. The higher p-distance value, the farther the relationship is. Furthermore, a coefficient of 0.20 hypothetical line is driven the common sunflower to be far from the three cultivars tested.

CONCLUSIONS

Based on the results of this study, it can be concluded that RAPD profile of Lemon Queen, Skyscraper, Teddy Bear sunflower cultivars and Common sunflower shows that Common sunflower has the most distinct characters from the other three cultivars. Genetic relationship among Lemon Queen, Skyscraper, Teddy Bear and Common sunflower based on RAPD markers shows that the cultivars studied are grouped into three main groups, namely: Group I Lemon Queen and Skyscraper, Group II Teddy Bear, and Group III Common sunflower; the closest relationship is shown between Lemon Queen and Skyscraper.

REFERENCES

Badouin, H., Gouzy, J., Grassa, C.J., Murat, F., Staton, S.E. Cottret, L. Lelandais-Brière, C. Owens, G.L. Carrère, S. & Mayjonade, B., 2017. The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. *Nature*, 546, pp.148-152.

Beard, B. & Geng, S., 1982. Interrelationships of morphological and economic characters of sunflower. *Crop Sci*., 22(1), pp. 817-822.

Darvishzadeh, R., 2016. Population structure, linkage disequilibrium and association mapping for morphological traits in sunflower (Helianthus annuus L.). *Biotechnology and Biotechnological Equipment*, 30(2), pp. 236-246.

Ellur, V., Goud, I. & Prabakaran, J., 2015. Morphological and molecular characterization of interspecific cross between cultivated Sunflower (Helianthus annuus L. with wild annual diploid H. argophyllus.). *Electronic Journal of Plant Breeding*, 7(2), pp. 377-389.

Griffin, H. & Annete, M., 1994. *PCR technonogy: current innovations*. 1st ed. America: CRC Press.

Hockett, E. & Knowles, P., 2018. Inheritance of branching in sunflowers, Helianthus annuus L. *Crop Sci*., 10, pp. 432-436.

Jannatdoust, M., Darvishzadeh, R., Ziaeifard, R., Ebrahimi, M.A., Maleki, H.H., Gholinezhad, E. & Hatamnia, A.A., 2016. Analysis of genetic diversity and population structure of confectionery sunflower (Helianthus annuus L.) native to Iran. *J. Crop Sci. Biotechnol*., 19(37), pp. 11-17.

Mandel, J.R., McAssey, E.V., Nambeesan, S., Garcia-Navarro, E. & Burke, J.M., 2014. Molecular evolution of candidate genes for crop-related traits in sunflower (Helianthus annuus L.). *Molecular*, 9(1), pp. 21-30.

Masvodza, D., 2015. Genetic Diversity Analysis of Local and Foreign Sunflower Germplasm (Helianthus annus) for the National Breeding Program : Zimbabwe. *Journal of Cereals and Oil Seeds*, 6(1), pp. 1-7.

Nascimento, A.M.P., Reis, S.N., Nery, F.C., Curvelo, I.C.S., Taques, T.C. & Almeida, E.F.A., 2016. Influence of color shading nets on ornamental sunflower development. *Ornam. Hortic.*, 22(1), pp. 101-106.

Premnath, A., Narayana, M., Ramakrishnan, C., Kuppusamy, S. & Chockalingam, V. 2016. Mapping quantitative trait loci controlling oil content, oleic acid and linoleic acid content in sunflower (Helianthus annuus L.). *Mol. Breed*., 36, pp. 106-112.

Smith, B., 2014. The domestication of Helianthus annuus L. (sunflower). *Veg. Hist. Archaeobot*., 23, pp. 57-74.

Sudrik, B.P., Ghodke, M.K., Patil, V.S., Chavan, S.K., & Kesale, N.B., 2014. Evaluation and characterisation of sunflower (Helianthus annuus L.) germplasm. *J Crop Weed*, 10(1), pp. 73-76.

Suresha, P., 2017. Original Research Article Genetic Diversity Analysis in Sunflower (Helianthus annuus L.) parental Lines Using SSR and RAPD Markers.. *Int.J.Curr.Microbiol.App.Sci*, 6, pp. 2069-2076.

Tomlekova, N., Kozgar, M. & Wani, M., 2014. *Mutation breeding for changed oil quality in sunflower. In Mutagenesis: Exploring Genetic Diversity of Crops.* 1 ed. Wageningen, The Netherlands: Wageningen Academic Publishers.

Vear, F., 2016. Changes in sunflower breeding over the last fifty years. *OCL*, 23, pp. 20-25.