Scientific Journal of University of Benghazi

SJUOB (2024) 37 (2) Applied Sciences: 93 – 100

Emsaed et al.

http://journals.uob.edu.ly/sjuob

Genetic Stability Assessment of Three Tomato Hybrids (*Lycopersicon esculentum*) grown in the Libyan Green Mountains Using SRAP and RAPD Techniques

Amani Emsaed [1] - Abdelrezk Elkagkheg [2] - Khaled Elmeer [3, *]

[1] *Agricultural and Animal Research Center, Al Bayda, Libya.*

[2] *Faculty of science, Al Qubah, University of Derna, Libya*

[3] *Horticulture Department, Faculty of Agriculture, University of Tripoli, Libya*

Received: 30 / 09 / 2024; Accepted: 05 / 11 / 2024

ABSTRACT

The genetic diversity of F1 tomato hybrid (Nada, Mona, and Dania) grown in Libyan Green Mountain (LGM) was investigated using SRAP and RAPD molecular marker techniques while the coefficient of variation C.V was used to investigate F1 seed purity. The coefficient of variation C.V., in the three cultivars Nada, Mona and Dania are 31.88, 51.66 and 44.18, respectively, indicating the homogeneity of Nada cultivar over Dania and Mona. The SRAP marker provided a total number of 29 amplified DNA bands with an average of 9.67 bands per primer, 26 of which were polymorphic (67% polymorphism), while the RAPD marker provided a total of 73 bands with an average of 14.6 bands per primer; all of them were 100% polymorphic. The genetic diversity for the three studied cultivars was 0.31, whereas Nada cultivar was the most homogeneous recording a genetic diversity of 0.24 compared with Mona 0.25 and Dania 0.28 Dania. Principal coordinate analysis (PcoA) divided tomato F1 hybrid samples into two main groups, the first (A) includes all the plants of a Nada hybrid cultivar, while group (B) consists of overlapping of Dania and Mona cultivars, reflecting the genetic relatedness.

KEYWORDS: Tomato hybrid, *Solanum Lycopersicon*, Genetic diversity, Genetic Stability, Seed purity.

1. INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is a highly valuable vegetable crop cultivated globally for both fresh consumption and processed products. It is a rich source of essential nutrients, particularly vitamins A and C, along with a variety of antioxidants.[1] Additionally, it holds substantial economic importance for producers and breeding industries across numerous countries.[2] Currently, approximately 7,500 cultivated tomato varieties exist worldwide, displaying considerable variation in size, shape, colour, and taste.[3] Traditional breeding methods face challenges like long breeding cycles, limited genetic diversity, and unpredictable results. These factors slow down the efficient development of new crop varieties with enhanced yields.[4] The strategic use of molecular markers in crop improvement programs enhances selection accuracy and shortens the breeding cycle enabling the faster development of new cultivars with desirable traits.[5]

The genetic purity of hybrid seed cultivars is very important in the process of producing hybrid seeds to meet the desires of the farmer, preserve his rights, and protect him from commercial fraud.[6]

***Correspondence**: Khaled Elmeer

K.Elmeer@uot.edu.ly

The contamination in the seeds may occur as a result of mixing with seeds resulting from the self-pollinating of the parents instead of pollinating him with the father to be crossed, due to negligence in the process of castration and removal of the anthers, or it may be due to the arrival of strange pollen grains to the line to be crossed with one of the well-known parents, Sometimes cheating is done intentionally by mixing the seeds of the first generation F1 as a result of their high price with seeds of the second generation F2.[7]

The purity of seeds in hybrid cultivars is confirmed by means of a test called (GOT) field Grow Out Test by planting replicates from the two parents involved in the composition of the hybrid cultivar with replicates of the hybrid cultivar. Morphological traits are studied and evaluated based on the similarity discrimination test and genetic stability (DUS), Distinctness, Uniformity and Stability. However, this method takes a long time and requires great effort and good experience.[8] The discovery of molecular biology techniques and the development of Molecular marker techniques identifying the genetic fingerprint of the parents of the hybrids and comparing them with the hybrid cultivar. In addition to the comparison within the hybrid cultivar for the identification of homology and genetic homogeneity.[8]

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

Among the markers that were used in the genetic purity test and the study of genetic stability of the hybrid cultivar are the techniques of RAPD as well as the SRAP technique, which was used in the test of the purity of tomato hybrids,[9] as well as in genetic stability and purity of tobacco hybrids.[8] A commonly utilized approach involves molecular markers, such as RAPD, which offer high-resolution insights into the genetic variation within tomato populations.[2] RAPD markers demonstrated effectiveness in distinguishing the studied lines by calculating the average Polymorphism Information Content (PIC) values.[10] The SRAP technique was used to study the genetic diversity of heat-tolerant tomato plants,[11] as well used to reveal the genetic relationship of tomato strains in Spain,[12], in addition, the technique was used to study the molecular variance of some quantitative traits in tomato fruit in F2 second generation plants.[13] This study aims to assess the purity and homogeneity of the F1 hybrid tomato plants grown in Libyan Green Mountain (LGM) (Fig. 1), which can be used to identify commercial fraud and determine the degree of homogeneity and purity of F1 hybrids.

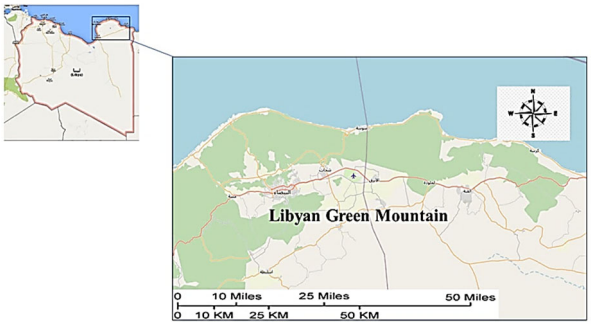

Figure 1 The Green Mountain region in Libya served as the site for the sampling studies conducted in this research.

2. MATERIAL AND METHODS

Thirty-three random samples of whole plants, including their fruits, were collected in late summer from the Libyan Green Mountain (LGM), an area characterized by a Mediterranean climate featuring warm to hot summer conditions and mild to cool winters. The samples included three F1 hybrid cultivars: Nada, Mona, and Dania (Fig 2).

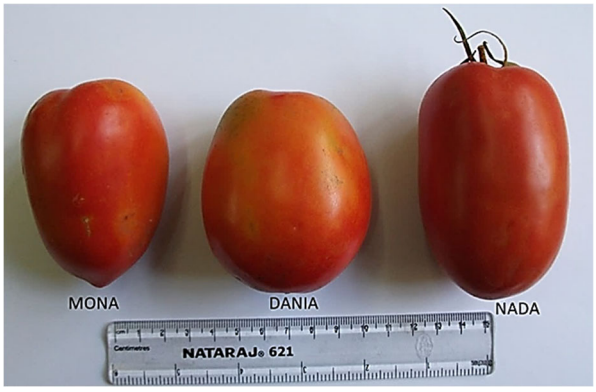

Figure 2: The F1 hybrid tomato varieties under investigation are Nada, Dania, and Mona.

The hybrids were sourced from TAJURI Agricultural Development Company located in Tripoli, Libya. for the study of morphological characteristics, eleven replicates were considered for each cultivar (Table 2). The data were analysed using ASSISTAT version 7.7 (2016), and mean comparisons were performed using Tukey's test ($p \leq 0.05$). Fresh, healthy leaves were washed with tap water followed by distilled water. Subsequently, the samples were disinfected using 70% ethyl alcohol. Finally, the samples were stored in plastic bags and placed in a freezer until DNA extraction.

One gram of each leaf sample was finely chopped and ground into a powder using washed, distilled, sterilized sand. Subsequently, an extraction solution was added following the CTAB extraction method. The isolated DNA was quantified and assessed by loading 2 µL onto a 0.85% agarose gel, which was run at 100 V for 30 minutes. The gels were then stained with ethidium bromide and visualized under ultraviolet light.

DNA amplification was conducted using a Px2 thermal cycler (Thermo) with eight primers, comprising three SRAP and five RAPD primers (Table 1)

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

Table 1. Sequences and melting temperature (Tm) of SRAP and RAPD primers

No.	Marker	Primer	Primer sequences (5'–3')	CG%	Optimal Tm °C
1	SRAP	me2	5' TGA GTC CAA ACC CGA GC – 3'	58.82	54.8
		em5	5' – GAC TGC GTA CGA ATT TGA – 3'	44.44	51.6
2	SRAP	me2	5' TGA GTC CAA ACC CGA GC – 3'	58.82	54.8
		em3	5' GAC TGC GTA CGA ATT TGC – 3'	50	53.9
3	SRAP	me13	5' – TGA GTC CAA ACC GGT TG – 3'	52.94	52.4
		em8	5' – GAC TGC GTA CGA ATT AAC– 3'	44.44	51.6
4	RAPD	OPA10	5' - GTG ATC GCA G – 3'	60	32
5	RAPD	OPC02	5' - GTG AGG CGT C – 3'	70	34
6	RAPD	OPC10	5' – TGT CTG GGT G – 3'	60	32
7	RAPD	OPC11	5' – GTG ATC GCA G – 3'	60	32
8	RAPD	OPD20	5' – ACC CGG TCA C – 3'	70	34

The conditions for the SRAP technique were as follows: initial denaturation at 94 °C for 5 minutes, followed by 5 cycles consisting of denaturation at 94 °C for 60 seconds, primer annealing at 35 °C for 60 seconds, and extension at 72 °C for 1 minute. This was followed by an additional 35 cycles of denaturation at 94 °C for 60 seconds, primer annealing at 47 °C for 60 seconds, and extension at 72 °C for 2 minutes, with a final extension at 72 °C for 10 minutes. For the RAPD technique, the protocol included an initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of denaturation at 94 °C for 45 seconds, primer annealing at the respective temperatures, and extension at 72 °C for 1 minute, concluding with a final extension at 72 °C for 5 minutes. Five microliters of the amplified DNA fragments, including a loading dye, were loaded onto a 1.5% agarose gel, which was run at 30 V for 180 minutes in 1X TAE buffer (30 mM). The gels were stained with ethidium bromide and visualized using a UV trans-illuminator.

SRAP (Sequence-Related Amplified Polymorphism) and RAPD (Random Amplification of Polymorphic DNA) bands were scored as present (1) or absent (0) to generate a binary data matrix. They were computed in PAST software V 1.91[14] to identify the relationships between the hybrids using Principal coordinate analysis (PcoA) through the hamming similarity index while analysis of molecular variance (AMOVA) assessment of genetic variation within and among populations was conducted using the GenAlex 6.5 software.[15]

3. RESULTS AND DISCUSSION

The statistical analysis presented in (Table 2) indicated significant variability in the fresh weight of both ripe and green fruits serving as a measure of plant productivity as determined by Tukey's test. The Nada hybrid exhibited the highest fresh fruit weight averaging 5609 grams per plant. In contrast, the Dania and Mona cultivars produced average weights of 3209 grams and 2421 grams, respectively, with no significant difference between these two cultivars. These variations in fruit weight may be attributed to the plants' responses to environmental factors and their genetic backgrounds.

Table 2 Productivity Traits and Coefficient of Variation CV of Nada, Mona and Dania F1 Hybrid Cultivars.

	NADA	CV	DANIA	CV	MONA	CV
Fresh fruit weight by gram	5609[a]	31.88	3209[b]	44.18	2421[b]	51.66
Number of fruits	85[a]	29.8	95.5[a]	37.12	42.5[b]	54.4
Fruit weight by gram	105.9[a]	9.6	83.18[b]	20.5	93.5[b]	14.3
Fruit size by mm3	111.3[a]	11.3	84.7[b]	26.7	81.8[b]	17.3
Number of locules	3.36[a]	15.52	2.61[b]	18.72	2.67[b]	17.71
Fruit rigidity by Durometers	7.2[a]	11.45	5.01[c]	18.92	6.76[b]	12.94

Each value represents the mean of eleven replicates (plants). Means followed by the same letter in each line are not significantly different by Tukey's test ($p \leq 0.05$).

The level of homogeneity within each cultivar serves as an indicator of the genetic purity of the hybrid cultivar. This homogeneity can be assessed using dispersion criteria. Given the differences in mean values of homogeneity among the cultivars, alternative measures of dispersion, such as standard deviation could not be employed. Nevertheless, the dispersion criteria were found to be consistent with the coefficient of variation.

The coefficients of variation for the three cultivars Nada, Mona, and Dania were 31.88, 51.66, and 44.18,

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

respectively (Table 2). These results indicate that the Nada cultivar demonstrates the greatest homogeneity exhibiting the smallest differences across all production traits (Table 2). In contrast, the Dania and Mona cultivars displayed notable dispersion and heterogeneity suggesting potential impurity. For comparison, a coefficient of variation of 14.3% for tomato fruit weight has been reported in Pakistan[16] indicating that hybrid cultivars can be differentiated based on vegetative vigor and homogeneity reflecting similar genetic backgrounds. When the coefficient of variation exceeds 30%, it typically indicates a high degree of heterogeneity with large genetic dispersion and minimal homogeneity among average values.[17,18] Furthermore, the coefficients of variation among genotypes significantly impact phenotypic traits, particularly productive traits such as fruit weight, in contrast to non-productive traits.[19]

In this study, the SRAP primers yielded 29 amplified DNA bands averaging 9.67 bands per primer with 26 of these being polymorphic resulting in a polymorphism rate of 67%. Conversely, a combination of the me2em3 and me13em8 primers achieved complete polymorphism (100%) while the me2em5 primer exhibited no polymorphism. The SRAP profiles of the amplified products from each primer are presented in (Table 3). The primer me2em3 produced the highest number of bands, totalling 14, whereas the primer me2em5 generated the fewest, with only three bands (Table 3). This number of bands surpasses that reported in a related study, which documented an average of 6 bands per primer among 15 tomato genotypes in Turkish breeding programs.[11]

Table 3. Amplified DNA bands and polymorphism percentage of SRAP and RAPD primers.

Marker	Primer	No of bands.	Polymorphic bands.	% Polymorphic	Genetic Diversity
SRAP	me2em3	14	14	100%	0.298501
SRAP	me2em5	3	0	0%	0
SRAP	me13em8	12	12	100%	0.308854
Average		9.67	8.7	67%	0.20
RAPD	OPA10	8	8	100%	0.314453
RAPD	OPC02	12	12	100%	0.309671
RAPD	OPC10	16	16	100%	0.413966
RAPD	OPC11	25	25	100%	0.339753
RAPD	OPD20	12	12	100%	0.263889
Average		73	14.6	100%	0.33
Both markers					**0.31757**

In a study involving 26 combinations of SRAP primers applied to local tomato cultivars in Spain,[12] a total of 384 bands were identified, resulting in an average of 14.77 bands per primer combination with a minimum of 4 bands and a maximum of 49. This variation in band number can be attributed to the diverse genetic compositions of the plants as well as the specific primers utilized. In the present study, the SRAP primer me2em3 (Fig. 3) produced profiles for 14 genotypes exhibiting a range of molecular weights with the heaviest band at approximately 1500 bp and the lightest at 250 bp found in the third sample of the Nada cultivar. Notably, the primers failed to amplify DNA in the first and second samples of the Nada cultivar. The same me2em3 primer combination previously applied to local tomato cultivars in Spain yielded only 4 bands,[12] while another analysis reported 10 bands with 70% polymorphism across different tomato cultivars.[20]

The SRAP primer me2em5 identified 3 genotypes with molecular weights ranging from 450 bp to 900 bp, while the primer me13em 8 detected 12 genotypes with molecular weights spanning from 50 bp to 1400 bp. Additionally, molecular weights between 500 bp and 1050 bp were recorded for the me2em3 primer.[11] In this study, the RAPD markers generated a total of 73 bands yielding an average of 14.6 bands per primer, all exhibiting 100% polymorphism. The RAPD profiles of the amplified products for each primer are detailed in (Table 4). In a separate study, an average of 9.7 fragments was identified using 16 RAPD primers across three tomato cultivars,[21] whereas another analysis employing 27 RAPD primers on 19 tomato cultivars reported a total of 442 RAPD bands, with an average of 16.4 bands per primer.[22]

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

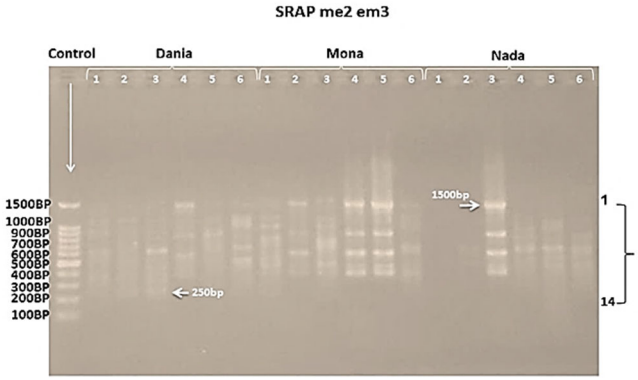

Figure 3: Banding patterns of F1 tomatoes hybrid obtained with me2 and em3 SRAP primers.

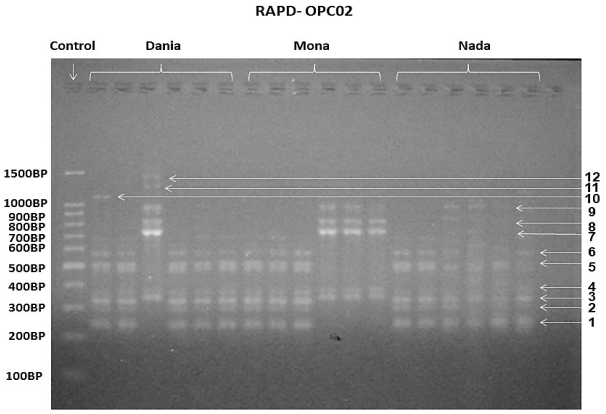

Figure 4: Banding patterns of F1 tomatoes hybrid obtained with OPC02 RAPD primers.

A maximum of 25 bands were amplified using the primer OPC11, while the primer OPA10 yielded a minimum of 8 bands. Additionally, the primers OPC02 and OPD20 each generated only 12 bands (Fig. 4). In previous research, the same primer was applied to various tomato cultivars and resulted in only 3 bands[23] of which two were polymorphic and one was non-polymorphic. Similarly, 7 bands were produced with the primer OPC11 in tomato cultivars from Korea; however, none of these bands exhibited polymorphism.[24]

All DNA fragments presented in (Table 3) were identified as sources of genetic variation among F1 hybrids of tomato cultivars exhibiting a polymorphism rate of 100%. In a study involving 24 tomato cultivars cultivated in India and examined with 11 RAPD primers, an average of 89.39% polymorphic bands was observed.[25] Conversely, an analysis of genetic relationships among eight tomato cultivars in Egypt, utilizing 7 RAPD primers, revealed an average of 11.57 bands per primer with 66.48% of these being polymorphic.[26] Additionally, a separate study identified an average of 4.14 bands per primer across 27 tomato cultivars in India with 63.81% showing polymorphism.[27]

The RAPD primer OPC10 produced the largest band, measuring 1600 bp, while the smallest band, at 200 bp, was generated by both the OPC10 and OPC11 primers. The primer OPC02 identified 12 genotypes with varying molecular weights (Fig. 4). The maximum molecular weight recorded was approximately 1400 bp corresponding to the third sample of the Dania cultivar, while the minimum was 250 bp, associated with 14 different samples across all cultivars. In a separate study, the same primer evaluated 11 tomato cultivars in Bangladesh yielding 41 bands with sizes ranging from 750 to 2000 bp.[28] Additionally, the same primer produced only 11 bands among 24 tomato cultivars in India.[25] These variations can be attributed to genetic differences among the cultivars studied resulting in distinct variations at the DNA level.

3.1. Genetic stability assessment using RAPD and SRAP:

The F1 hybrid tomato cultivars were distinguished by their high quality, productivity, early ripening, uniformity of fruit, and disease resistance. However, the production of F1 seeds presents significant challenges, as it demands highly skilled labour and precise knowledge of production techniques. The evaluation of hybrids typically takes into account factors, such as the characteristics of the parent plants, the male-to-female ratio, the precision of emasculation, and the compatibility of pollination. Additionally, any plants that do not meet the standards of the parental lines must be eliminated, as they are required to be completely identical and pure (100%). Inaccurate execution or omission of any essential procedures may result in the generation of impure or pseudo-hybrid genotypes.[29] The genetic diversity among the three studied cultivars was measured at 0.31, indicating low genetic diversity. Nevertheless, this value for genetic stability was consistent with the observed genetic diversity (0.31), in comparison to a previous study involving 25 hybrid cultivars from Italy.[30]

Among the cultivars, the Nada cultivar exhibited the highest homogeneity in genetic diversity at 0.24, followed by Mona at 0.25, and Dania at 0.28. Dania displayed the lowest homogeneity due to its higher genetic diversity, which may be linked to seed purity issues. A genetic diversity index of 0.41 was reported for 335 tomato strains collected from 25 Asian countries, evaluated using SSR markers,[31] although fourteen genotype pes of tomato cultivars in Azerbaijan tested using 6 RAPD primers, the genetic diversity rate was 0.61,[32] it may be due to samples are collected from different countries, environments, and sources compared with our study. Understanding the genetic

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

distance among diverse cultivars is crucial for plant breeders, as it facilitates the selection of suitable parent plants for developing new genetic combinations

Principal coordinate analysis (PcoA) divided tomato F1 hybrid samples into two main groups, the first includes all the plants of a Nada hybrid cultivar, which appear as group A, while the samples of the cultivar Dania and Mona overlapped with each other in group B (Fig. 5). It is an indicator that reflects the genetic affinity between the plants of the two cultivars Dania and Mona, which may be caused by the closeness of the parents used in the crossbreeding process. It also indicates greater homogeneity in the cultivar Nada compared to the other two cultivars.

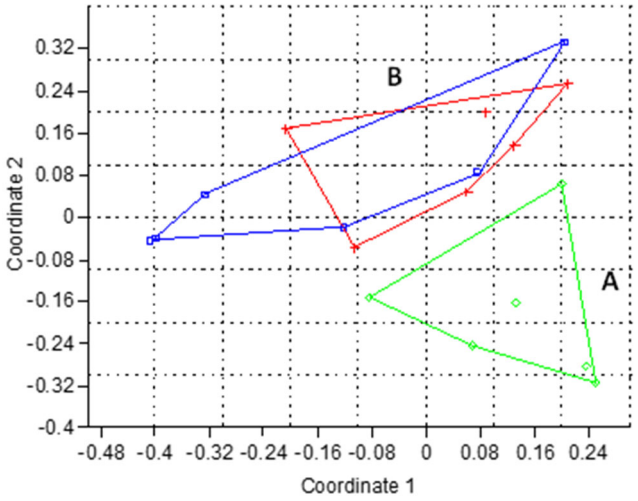

Figure 5: Scatter plot of Principal Coordinate Analysis (PCoA) of RAPD and SRAP markers divided tomato F1 hybrid into two main groups A: (Nada hybrid) and B: (Dania and Mona hybrids).

This finding coincided with the study on different genotypes of tomato in Sudan using 16 primers of the RAPD marker, where closely related strains were grouped into separate groups.[21]

Analysis of molecular variance (AMOVA) shows that only 16% of the variance was between the three different cultivars, while 84% of the genetic variance is attributed to differences within the cultivars (Fig. 6). This is an indication the lack of genetic purity, which may be caused by either error in the emasculation process, which caused self-pollination of mothers, or it may be contaminated with pollen from other cultivars.

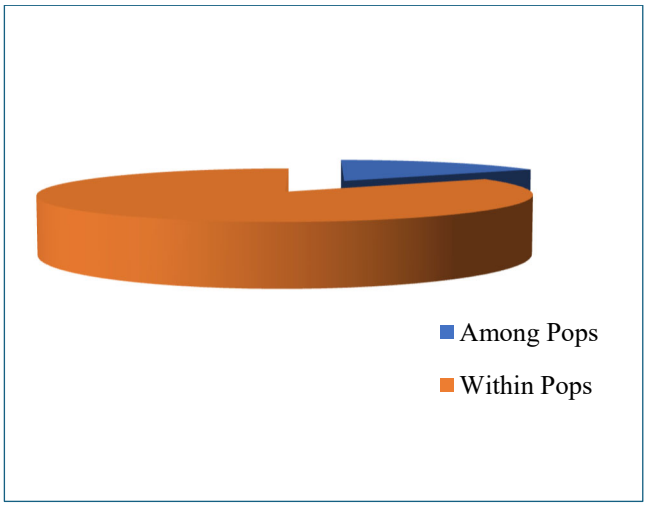

Figure 6: Analysis of molecular variance (AMOVA) of the three F1 hybrid tomato cultivars.

The DNA molecular marker was used to test seed genetic purity of two commercial F1 hybrid cultivars 'Hezuo 903' and 'Sufen No. 8' showed that eight of the 210 F1 plants in 'Hezuo 903' and 13 of 210 in 'Sufen No. 8' were false hybrids.[10]

4. CONCLUSION

This study is considered one of the first studies to identify the common genotypes of tomato F1 hybrids in (LGM) using DNA molecular markers, which could have strong implications for the breeding programs to develop and improve tomatoes as a commercially important crop and will be useful for future programs on genetic improvement of tomato cultivars. The genetic stability for the studied tomato cultivars was appropriate, the cultivar Nada is the most homogeneous through phenotypic and genetic results, while the Mona cultivar is the least homogeneous, which may be attributed to the lack of purity of seeds. This result was proved by the Principal coordinate analysis (PcoA) test, which separated the most homogeneous cultivar into one group, while the rest of the cultivars were in the second group. This adulteration of the seeds may be due to errors in the production process, or it may be deliberate by some fraudsters, which requires the state to enact deterrent laws for that matter.

REFERENCES

1. Tiwari JK, Rai N, Singh MK, Mishra LK, Mishra G, Behera TK. Characterization of tomato lines for yield components, processing traits and disease resistance based on phenotype and molecular markers. Vegetable Science. 2023 Dec 31;50(2):302-9.

2. Umar YI, Onovo JC, Alanana JA, Abubakar NA. Assessment Of Genetic Variability Of Three Tomato Genotypes (Lycopersicon Esculentum L.) Using Phenotypic Parameters And Genetic Markers. Advance

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018

Journal of Agriculture and Ecology. 2024 Jun 14;9(6):8-21.

3. Ahang B, Pashapour S, Ghasemi A, Zabihi A. Investigating the genetic diversity of several varieties of Iranian tomato (Solanum lycopersicum) using RAPD and ISSR molecular markers. Food Health. 2024; 6 (4): 18. doi: 10.53388/FH2024018. Executive editor: Nuo-Xi Pi. Received. 2024 Aug;9.

4. Khan AA, Iqbal B, Jalal A, Khan KA, Al-Andal A, Khan I, Suboktagin S, Qayum A, Elboughdiri N. Advanced Molecular Approaches for Improving Crop Yield and Quality: A Review. Journal of Plant Growth Regulation. 2024 Mar 2:1-3.

5. Mapari AR, Mehandi S. Enhancing Crop Resilience: Advances and Challenges in Marker-Assisted Selection for Disease Resistance. Journal of Advances in Biology & Biotechnology. 2024 Jun 19;27(7):569-80.

6. Afzal I, Shabir R, Rauf S. Seed production technologies of some major field crops. Agronomic Crops: Volume 1: Production Technologies. 2019:655-78.

7. Battistini E, Teriaca M, Noli E. A Quantitative Real-Time Approach For Testing Trait Purity In Transgenic Varieties. InProceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress 2009 Sep (Vol. 16, p. 19).

8. Kumar DS, Kalyani D. Genetic Purity of Flue Cured Tobacco F1 Hybrid with RAPD Markers. International Journal of Current Microbiology and Applied Sciences. 2016;5(2):557-66.

9. Wang Y, Gong Y, Zhai X, Yu F, Shen H, Liu L. Genetic Purity Test of F1 Hybrid Tomato Using Molecular Marker Analysis. InXXVII International Horticultural Congress-IHC2006: International Symposium on Seed Enhancement and Seedling Production 771 2006 Aug 13 (pp. 231-238).

10. Anwar A, Roy S, Islam MN. Genetic variability analysis of tomato varieties using rapd markers under heat stress. Bangladesh Journal of Botany. 2024 Mar 31;53(1):91-100.

11. Comlekcioglu N, Şimşek ÖZ, Boncuk M, Aka-Kacar YI. Genetic characterization of heat tolerant tomato (Solanum lycopersicon) genotypes by SRAP and RAPD markers. Genetics and molecular research. 2010;9(4).

12. Ruiz JJ, García-Martínez S, Picó B, Gao M, Quiros CF. Genetic variability and relationship of closely related Spanish traditional cultivars of tomato as detected by SRAP and SSR markers. Journal of the American Society for Horticultural Science. 2005 Jan 1;130(1):88-94.

13. JH PD, GR R, DR L, SL M, Basté E M, GR P. Tomato second cycle hybrids as a source of genetic variability for fruit quality traits. Crop Breeding and Applied Biotechnology. 2016 Oct;16:289-97.

14. Hammer Ø, Harper DA. Past: paleontological statistics software package for educaton and data anlysis. Palaeontologia electronica. 2001;4(1):1.

15. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics. 2005 Jan;1:117693430500100003.

16. Saleem MY, Iqbal Q, Asghar M. Genetic variability, heritability, character association and path analysis in F1 hybrids of tomato. Pak. J. Agri. Sci. 2013 Dec 1;50(4):649-53.

17. Brown CE. Coefficient of variation. InApplied multivariate statistics in geohydrology and related sciences 1998 (pp. 155-157). Berlin, Heidelberg: Springer Berlin Heidelberg.

18. Taylor SL, Payton ME, Raun WR. Relationship between mean yield, coefficient of variation, mean square error, and plot size in wheat field experiments. Communications in Soil Science and Plant Analysis. 1999 May 1;30(9-10):1439-47.

19. Semel Y, Nissenbaum J, Menda N, Zinder M, Krieger U, Issman N, Pleban T, Lippman Z, Gur A, Zamir D. Overdominant quantitative trait loci for yield and fitness in tomato. Proceedings of the National Academy of Sciences. 2006 Aug 29;103(35):12981-6.

20. Ramesh SM. Stability analysis in tomato (Solanum lycopersicum Mill.) genotypes (Doctoral dissertation, UAS, Dharwad, 2009).

21. Aida AE, Eltayeb EA. DNA polymorphism of three tomato (Solanum lycopersicum) landraces from Sudan using RAPD markers. International Journal of Current Microbiology and Applied Sciences. 2015 May 13;4(4):1-8.

22. Al-Saadi AH, Abbass MC. Genetic Diversity of Some Tomato Lycopersicon esculentum Mill Varieties in Iraq Using Random Amplified Polymorphic DNA (RAPD) Markers. Journal of University of Babylon. 2014;22(9).

23. Mavromatis AG, Athanasouli V, Vellios E, Khah E, Georgiadou EC, Pavli OI, Arvanitoyannis IS. Characterization of tomato landraces grown under organic conditions based on molecular marker analysis and determination of fruit quality parameters. Journal of Agricultural Science. 2013 Feb 1;5(2):239.

24. Huh MK, Youn SJ, Kang SC. Identification and genetic diversity of Korean tomato cultivars by RAPD markers. Journal of Life Science. 2011;21(1):15-21.

25. Rajendra Prasad S, Prasanna KP. Varietal characterization of tomato cultivars based on RAPD markers. Research Journal of Agriculture and Biological Sciences. 2010;6(6):713-5.

26. Abd El-Hady EA, Haiba AA, Abd El-Hamid NR, Rizkalla AA. Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. Journal of American Science. 2010;6(11):434-41.

27. Archak S, Karihaloo JL, Jain A. RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Current Science. 2002 May 10:1139-43.

28. Tabassum N, Sony SK, Bhajan SK, Islam MN. Analysis of genetic diversity in eleven tomato (Lycopersicon esculentum Mill.) varieties using RAPD markers. Plant Tissue Culture and Biotechnology. 2013 Jul 3;23(1):49-57.

29. Hashizume T, SATo T, HIRAI M. Determination of genetic purity of hybrid seed in watermelon (Citrullus lanatus) and tomato (Lycopersicon esculentum) using random amplified polymorphic DNA (RAPD). Japanese Journal of Breeding. 1993 Sep 1;43(3):367-75.

30. Corrado G, Caramante M, Piffanelli P, Rao R. Genetic diversity in Italian tomato landraces: Implications for the development of a core collection. Scientia Horticulturae. 2014 Mar 26;168:138-44.

31. Raveendar S, Lee GA, Lee KJ, Shin MJ, Cho YH, Ma KH, Lee JR. Genetic Diversity and Population Structure of Asian Tomato Accessions Based on SSR Marker. 2016 Jun;2016(1):152-.

32. Sharifova S, Mehdiyeva S, Theodorikas K, Roubos K. Assessment of genetic diversity in cultivated tomato (Solanum lycopersicum L.) genotypes using RAPD primers. Journal of Horticultural Research. 2013;21(1):83-9.

 ISSN:Online 2790-1637, Print 2790-1629; National Library of Libya, Legal number : 154/2018