International Journal of Biochemistry Research & Review
16(2): 1-9, 2017; Article no.IJBCRR.32097
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Study of Genetic Diversity among Different Tomato (*Lycopersicum esculentum* Mill.) Lines through Morphological and Biochemical Analysis

Qumqum Noshad[1], Muhammad Ajaib[1*], Nafees Iqbal[1], Nafeesa Zahid[1], Sadia Jehangir[1] and Neelam Rashid[1]

[1]Department of Botany, Mirpur University of Science and Technology (MUST), Bhimber Campus, Mirpur-10250 (AJK), Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Author QN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MA and NI managed the analyses of the study. Authors NZ, SJ and NR managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/32097
Editor(s):
(1) Kuo-Wei Lin, Department of Food and Nutrition Providence University, Taiwan.
Reviewers:
(1) Hakan Sevik, Kastamonu University, Turkey.
(2) Hatice Pasaoglu, Gazi University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/18474

Original Research Article

Received 8th February 2017
Accepted 14th March 2017
Published 3rd April 2017

ABSTRACT

Aims: The aims of this study were to characterize the existing genetic diversity from Azad Jammu & Kashmir and to identify some selectable markers based on total leaf protein profiles. The purpose of the investigation was to detect any possible variations among selected cultivars and to document the protein profiling for future record. The total leaf proteins profile based on SDS-PAGE showed bands of lower molecular weight common among all the varieties.
Study Design: Study was conducted on the basis of randomized complete block design.
Place and Duration of Study: Sample: Field experiment was conducted in different plots in all three districts Mirpur, Bhimber and Kotli and lab experiment was conducted in Department of Botany Mirpur University of Science and Technology during the year 2012-2014.
Methodology: Please write main points of the research methodology applied. Plant sample such as

**Corresponding author: E-mail: majaibchaudhry@yahoo.com;*

1 Red wonder 2 Tomato Galia 3 Rizwan tomato Super special -F1 4 Tomato Red King -F1 5 Tomato 117-F1 collected and Following parameters were studied Morphological analys Biochemical analysis by SDS PAGE and Statistical analysis was performed.

Results: Results was confirmed through cluster analysis, principal component analysis and Minitab. Dendrogram was achieved from the cluster analysis of five varieties of *Lycopersicum esculentum* was based on five morphological traits. Distance estimated based on five morphological traits ranged from 6-60 with an average of 33 Proteins distribution patterns of fourteen cultivars of *Lycopersicum esculentum* disclose there are significant variations in terms of banding pattern. Analysis of gel electrophorogram of SDS PAGE showed that there are significant differences between the bands of all fourteen cultivars. There were total fourteen bands present in each tomato variety the molecular weight of marker ranges between 10-200 kDa. There were two bands No. 12 and 14 common among all fourteen cultivars. These bands are of low molecular weight with the molecular weight of 20 and 10 kDa, respectively.

Conclusion: It is concluded from present work that genetic diversity existed among all the cultivars. On the basis of all parameter studied, it was found that Red wonder is the best among all lines.

Keywords: Morphological analysis; Lycopersicum esculentum; genetic diversity; SDS PAGE; biochemical analysis.

1. INTRODUCTION

Solanaceae is a family of about 94 genera and more than 2950 species, also called as nightshades and distributed in most part of the world, more especially in tropical America [1]. In Pakistan it is represented by 14 genera and 50 species [2]. Pakistan is producing fresh tomato of about 0.47 MT and at the 34th position in fresh tomato production world widely [3]. The world financial records for vegetables tomato rank first in 14% of world vegetable production (Food and Agriculture Organization) [4]. Tomato is grown in almost all over the world. High production and consumption of tomato is the major reason for the tomato processing industry. Its worldwide production reached about 126 million tones in 2007 [5]. The collection and assessment of genetic diversity must be advantageous to build up better methods for assessment and conservation of tomato germplasm resources. The present study attempted, therefore, to compare the local *Lycopersicon* germplasm and its relatives growing in different agro-ecological zones of Azad Jammu and Kashmir by using protein markers. The main task of this work is to assess the genetic variations in some tomato varieties of indigenous collection, based on leaf protein patterns [6]. Morphological characteristics are precious for the determination agronomic value and taxonomic classification of plants. The consistency of scope can be improved by repeating experiments in plentiful environments. The genetic variability is the raw material of vegetable breeding industry on which selection acts to develop superior genotypes. Greater the eminent amount of variation present for a character in the breeding materials, greater is the aptitude for its enhancement through selection. In tomato, yield is the snowballing effect of many ingredient characters individually contributing towards yield [7].

In recent years, SDS-PAGE of total seed proteins and psychological analysis has been found large application in resolving genetic diversity and for intra and inter specific studies [8].

The electrophoretic protein profiles and their high fidelity and independence of the ecological conditions could be used as genotypic markers in coming future for analysis of protein profiling of several plants. These genetic markers are also extensively used as tackle for the identification and inference of the quantitative traits in plant resources, resistant to disease and environmental hassle conditions, and other desirable agronomic traits [9]. Statistical tools are extensively used in plant propagation and inheritance to study genetic variability in a succession of breeding supplies. This is one of the superior techniques for the measurement genetic diversity [10]. These statistical methods embrace principal component analysis (PCA) and cluster analysis, which have equivalent competence to set up the most appropriate cross combinations [11]. To find out the family relationships cluster analysis is a appropriate method [12]. The basic purpose of cluster analysis is to expand the homogeneity within clusters and increasing heterogeneity between clusters [13]. The PCA has preference over the cluster analysis in respect to having

distinguishing character that by the PCA each genotype can be assigned to one group only [14].

2. MATERIALS AND METHODS

2.1 Plant Sample

Seeds of five tomatoes lines were collected from agriculture research centers. The samples were stored in labeled glass bottles to ensure safety and grown at Dadyal, Pothi and Nakyal of Districts Mirpur, Bhimber and Kotli respectively. The varieties were:

1. Red wonder
2. Tomato Galia
3. Rizwan tomato Super special -F1
4. Tomato Red King -F1
5. Tomato 117-F1

Following parameters were studied

Morphological analysis
Biochemical analysis by SDS
Statistical analysis

2.2 Field Experiment

Seeds were sown in five plots by using furrows on lines with same length and width on the basis of randomized complete block design [15] without the addition of fertilizers in sampling plot respectively then seedling shifted to different plots in all three districts Mirpur, Bhimber and Kotli.

2.3 Morphological Analysis

Plants were selected at random for five agronomic traits as follows:

Plant height before shifting and at maturity (cm), area of leaves (cm²), weight of leaves/ plant (g), no of flowers/ plant, no of fruits /plants [16].

2.4 Biochemical Analysis (SDS-PAGE)

The study of genetic diversity of *Lycopersicum esculentum* was done by using the sodium Dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The present work was the count (3) of preceding work morphogenetic comparison of three tomato cultivars from Azad Jammu and Kashmir, Pakistan [17].

2.4.1 Plant material

Leaves of all five tomatoes were crushed to powder form and 0.1 g from each sample was weighed to 1.5 ml micro tubes.

2.4.2 Extraction of protein

Protein extraction and protein analysis was done by following the Furdi Florina, [9]. SDS-PAGE is a significant and momentous method for the identification of whole cell proteins at species level. Molecular weights of leaves proteins are analyzed by SDS-PAGE by using Fermentas protein ladder SM0661 for SDS Gel Electrophoresis the molecular weight of the fermentas SM0661 ranged between 10kDa to 200kDa.

2.4.3 Preparation of resolving gel (10% acryl amide gel)

The separating gel was lay down by mixing 3 ml (1.875M -HClPh 8.80), 6.9 ml distilled water, 5 ml (5% acryl amide), 140 µl (SDS 10%), 90 µl (APS 5%) and 14 µl TEMED.

2.4.4 Preparation of stacking gel

Mixed 1 ml (0.6 M Tris-HCl pH 6.8), 7.2 ml distilled water, 1.66 ml (30% acryl amide), 100 µl (SDS 10%), 80 µl (APS 5%) and 9 µl (TEMED) at the end.

2.4.5 Gel preparation

Glass plates were cleaned with 70% ethanol and fixed by using seal gasket and clips; separating gel was poured to the cell and layered with water. After 30 min distilled water was removed, stacking gel was added and comb was inserted into the stacking gel [17].

2.4.6 Sample loading and electrophoresis

Glass cabinet was fixed with electrophoresis apparatus and filled the electrophoretic trays with electrode buffer (25 Mm Tris, 0.1%SDS, 192 mM glycine). The wells was clean with running buffer, and the sample (12 ul) and molecular weight marker (10-200 kDa, Fermentas protein ladder) (5 µl) was loaded at the bottom of each well using micropipette and connected the power supply at 120 volts [17].

2.4.7 Staining

After electrophoresis the gel was shifted to tray containing staining solution which was composed

of trichloroacetic acid solution, ethanol, glacial acetic acid and Coomassie Brilliant Blue (CBB), and was shaken gently for 40 min.

2.4.8 De-staining

Staining was followed by distaining solution containing methanol, acetic acidand distilled water and kept over night until the background of gel disappeared. The picture was taken by gel documentation with white light illuminator [18].

2.4.9 Data scoring

Electrophoregram for each variety was scored and the presence (1) or absence (0) of each band was renowned. One the basis of electrophoresis band spectra Jacquard's similarity index was calculated by using formula [19]. The scoring was done by analyzing the presence or absence of data regarding the banding patterns on the gel of the leaf storage proteins of tomatoes [20].

2.5 Statistical Analysis

The morphological character was analyzed using cluster analysis, principal component analysis and Minitab. Correlation between morphological as well as biochemical traits was done by using the statistical tools. By using the procedure of cluster analysis, principal component analysis, and Minitab computer software programs for windows, data was analyzed statistically [19].

3. RESULTS AND DISCUSSION

Genetic diversity is the richness of the hereditary information in the gene pole of one species. High level of inter-species genetic diversity is an assurance for adaptation to changing environmental conditions, an indication for adaptation potential of the species and an important part of the ecosystem stability. Also genetic diversity is a raw material for tree improvement studies. As such, most of the researches about the genetic diversity are in high priority in plants improvement programs [25-28]. Genetic diversity can be determined by morphological and physiological characters or molecular markers. Morphological and physiologic which is especially enough to get information after obtaining to reach the details of information of izoenzim and DNA studies [29-32]. For this, there are very much studies about genetic diversity on the different plant species [33,34].

Dendrogram was achieved from the cluster analysis of five varieties of *Lycopersicum esculentum* was based on five morphological traits. Distance estimated based on five morphological traits ranged from 6-60 with an average of 33 (Fig. 1). Cluster analysis based on morphological traits clustered the genotypes in to two main clusters. The first cluster included the Tomato Red King-F1 line, and the second cluster was divided into two sub groups on the basis of similarity and differences. Results showed that all four lines were originated from the singleton. The first sub-group included the Rizwan tomato Super special -F1 and Tomato 117-F1, and the second sub-group comprised of Red wonder and Tomato Galia that conferred the genetic similarity among them.

PCA bi-plots provided an indication of the similarities and variation between the genetic diversity among different accessions of the same genotype and interrelationships between all genotypes. The PC1 and PC2 cumulatively explained variation among five lines of *Lycopersicum esculentum* (Fig. 2). All the five genotypes grown in three districts remained scattered in all quadrants, showing great genetic inconsistency particularly Red wonder which was on top right of ordination plot while Tomato Red King–F1 line was on top left of the ordination plot.

In the top two quadrants of the projection both the lines Red wonder, and Tomato Red King –F1 were scattered and showed the variation between these two lines. In lower right quadrant of projection Tomato Galia was present showing the trend of genetic divergence. Lower left quadrant of projection Tomato 117-F1 and Rizwan Tomato superspecial-F1 lines were present. They were almost overlapping in lower quadrant of projection that represented these lines were genetically similar in relationship.

3.1 Biochemical Analysis by SDS PAGE

Proteins distribution patterns of fourteen cultivars of *Lycopersicum esculentum* disclosed that there were significant variations in terms of banding pattern. Analysis of gel electrophorogram of SDS PAGE showed that there were significant differences between the bands of all fourteen cultivars. There were total fourteen bands present in each tomato variety and the molecular weight of marker ranged between 10-200kDa. There were two bands (No. 12 and 14) common (meaning?) among all fourteen cultivars. These

bands are of low molecular weight with the molecular weight of 20 and 10kDa respectively. (Fig. 3)

Similarity coefficient matrix obtained from the fourteen lines of *Lycopersicum esculentum* grown in all three areas represented that coefficient of similarity ranged between 0.4-0.96296 with an average value of 0.5. The minimum similarity was observed between Tomato Red King-F1 grown at Mirpur and Red Wonder grown at Kotli with the value 0.4. The maximum similarity was observed between the Tomato Red King-F1 grown at Kotli and Tomato Gali grown at Kotli.

Dendrogram was obtained from the cluster analysis of five lines grown in all three districts of *Lycopersicum esculentum* based on banding pattern on gel as well as similarity coefficient matrix. Distance estimated based on genetic similarity ranged between 0.0-3.0 with an average mean value of 1.5. Cluster analysis based on genetic similarity clustered the genotypes into three main clusters. The first cluster included two sub group the first subgroup included three cultivars Red Wonder (M), Tomato Galia (M), and Rizwan tomato super special-F1 (M). Where as second subgroup was singleton with Tomato Red King-F1 (M) showed the trend towards the origination of all remaining lines from cluster-one. Second cluster was also divided into two subgroups. First sub group comprised of singleton cultivar Tomato117-F1 line from district Bhimber. Second sub group divided into two subgroups, second subgroup was consisted of three lines Rizwan tomato super special-F1 (B) and Tomato Red King-F1 (B) and Tomato Galia (B) was singleton. Third cluster also clustered into two subgroups. First subgroup was comprised of three lines singleton with the cultivar Red Wonder (B) while other two were Red Wonder (K) and Rizwan tomato super special-F1 (K) where as second sub group consisted of a singleton and a sub group. Singleton Tomato 117-F1 (M) and Tomato Red king-F1 and Tomato Galia both grown at Kotli showed their trend of origination from singleton Tomato 117-F1 (M) (Fig. 4).

The Principal Component Analysis (PCA) tool was used to analyse the data obtained through banding pattern, similarity coefficient matrix and cluster analysis of all fourteen lines of tomato grown in three localities (Fig. 5).

PCA bi-plots provided an indication of the similarities and variation between the genetic diversity among different accessions of the same genotype and interrelationships between all genotypes. The PC1 and PC2 cumulatively explained variation among fourteen lines of *Lycopersicum esculentum* (Fig. 4). Projection of all genotypes on a two-dimensional plane, based on the first two PCs, partially confirmed the results of dendrogram (Table 2).

In the top right quadrant of ordination Red wonder (K), Tomato Galia (K), Rizwan tomato super special -F1 (K), Tomato Red King -F1 (K) and Tomato 117-F1 (M) lines were forming a lose group represented that all thelines were genetically similar. In the top left quadrant of ordination Red wonder (M), Tomato Galia (M) and Rizwan tomato Super special-F1 (M) were forming a compact group represented strong genetic relationship among all three lines of quadrant. In lower right quadrant of projection Red wonder (B), Tomato Galia (B) and Tomato 117-F1(B) formed group by showing a overlapping pattern. While on the lower left quadrant of projection Tomato Red King-F1 (B) and Rizwan tomato Super special -F1 (B) were present showing that all these tomato lines in lower both quadrants showed almost overlapping which means these were genetically similar except the Tomato Red king–F1 (M) that was far away from other tomato lines showing that it was not similar to other tomato lines. (Separate into two or three grammatically correct sentences for better clearification).

Morphological trait measurements can provide a simple method of estimation of genetic variation while harmoniously assessing genotype presentation under related growing environments [20]. Morphological and molecular data supported self incompatibility (self pollen rejection), cross fertilization in plants (allogamy), and green fruits as plesiomorphic in tomatoes [21]. Though, evaluation of morphological character is protracted and in general phenotypic characters are dependent mostly upon plant developmental stages and environmental conditions.

Comparison was based on leaf protein banding patterns in SDS PAGE. The banding patterns showed two common bands among all tomato cultivars. The bands of low molecular weight 14 and 12 were present in all and recorded among all fourteen tomato lines. These proteins were of molecular weight of 10 kDa of band No. 14 which may be the xylem sap proteins as reported in the previous work of Rep et al. [22]. The other low molecular weight proteins of band No. 14 may be

heat shock proteins as having molecular weight of 20 kDa as reported previously by Ding et al. [23]. The intensity of variations depends upon the glance of the bands. However banding patterns of major bands obtained did not show variability but there were some variations among the minor bands. No work had been reported previously about the leaf proteins analysis of the *Lycopersicum esculentum* by SDS-PAGE. The present work would be a support for future analysis.

For the achievement of success in breeding programs the information of relationship of fruit yield with its constituent traits helps greatly. Singh et al. [24] reported high genetic divergence for plant height, number of days to fruit set, number of fruit clusters per plant, number of fruits per plant, fruit weight per plant and fruit yield per plant. The level of relationship between characters with yield can be established by studies of correlation. This would help in formulation of a competent breeding program for improving the yield prospective by means of its components. This work will be helpful to farmers for cultivation of these varieties in particular areas. The genetic inconsistency proved to be a basic material of vegetable breeding industry on which selection acts to expand better genotypes. The elevated quantity of divergence present for a character in a genotype favors greatly easy selection of genotype for breeding and improvement of the crop plant in future. In tomato, yield was the snowballing effect of many constituent characters independently causative towards yield.

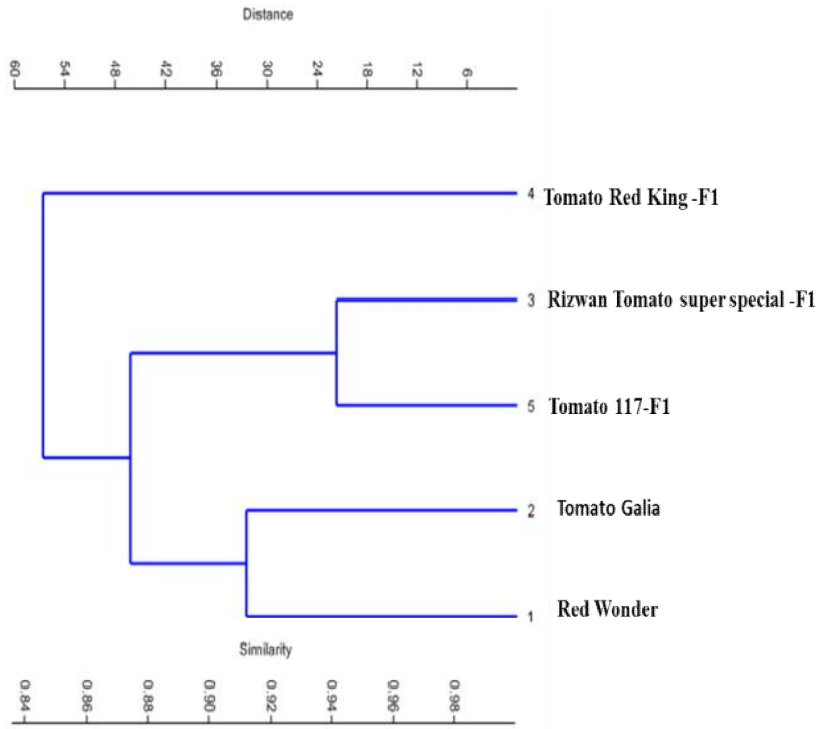

Fig. 1. Dendrogram based upon morphological traits

Table 1. Similarity index based upon morphological traits

Species name	Red wonder	Tomato Galia	Rizwan T -F1	Tomato RK -F1	Tomato 117-F1
Red wonder	1				
Tomato Galia	0.98458	1			
Rizwan T -F1	0.96128	0.98282	1		
Tomato RK -F1	0.86228	0.85319	0.82262	1	
Tomato 117-F1	0.95629	0.97414	0.986	0.86494	1

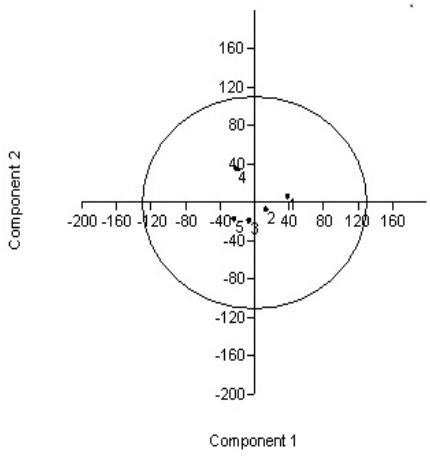

Fig. 2. PCA of *Lycopersicum esculentum* based upon morphological trait

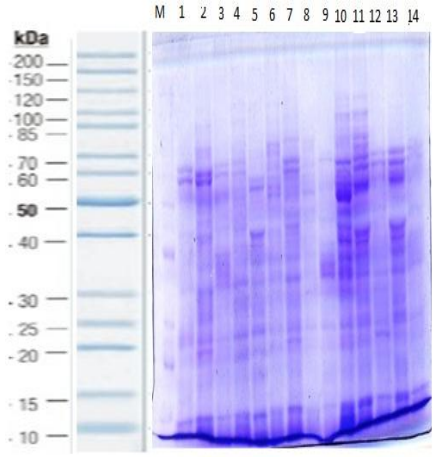

Fig. 3. Gel obtained from SDS-PAGE

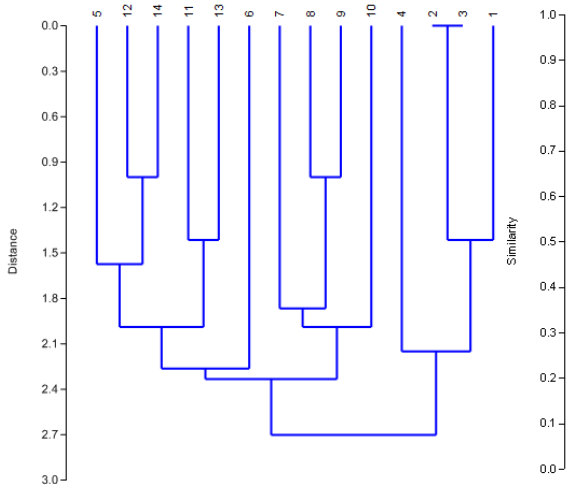

Fig. 4. Dendrogram based upon SDS-PAGE analysis

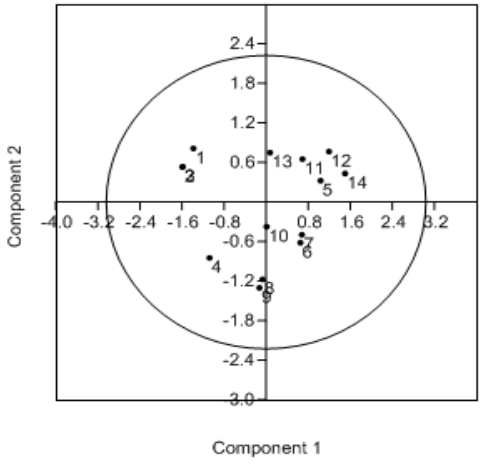

Fig. 5. PCA based upon SDS-PAGE analysis

Table 2. Index of similarity obtained from SDS –PAGE

Species name	Red wonder	Tomato Galia	Rizwan T -F1	Tomato RK -F1	Tomato 117-F1
Red Wonder	1				
Tomato Galia	0.75	1			
Rizwan T -F1	0.75	0.5	0.5		
Tomato RK -F1	0.25	0.5	1	1	1
Tomato 117-F1	0.5	0.5	0.5	0.5	1
Red Wonder	0.30769	0.30769	0.61538	0.85714	1
Tomato Galia	0.42857	0.42857	0.42857	0.81818	0.73684
Rizwan T -F1	0.33333	0.5	0.66667	0.7	0.70588
Tomato RK -F1	0.36364	0.36364	0.54545	0.63158	0.625
Tomato 117-F1	0.46154	0.61538	0.61538	0.85714	0.66667
Red Wonder	0.53333	0.53333	0.4	0.8695	0.7
Tomato Galia	0.47509	0.78261	0.8	0.83333	0.72727
Rizwan T -F1	0.61538	0.66667	0.9	0.81818	1
Tomato RK -F1	0.44444	0.92308	0.96296	0.78261	1

4. CONCLUSION

It is concluded from the present work that genetic diversity existed among all the cultivars. On the basis of all parameter studied it was found that Red wonder is the best among all lines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mabberley DJ. The plant book. Cambridge University Press. 1987;707.
2. Nasir JY. Solanaceae. In: Flora of Pakistan. (Eds.): SI. Ali and E. Nasir. Fascicle Pakistan Agricultural Research council, Islamabad. 1985;168:1-61.
3. Rizvi SMD, Shakil S, Haneef M. A simple click by click protocol to perform docking: Autodock 4.2 made easy for non-bioinformaticians. EXCLI J. 2013;12:831–857.
4. FAO, FAOSTAT. Food and Agriculture Organization of the United Nations; 2010.
5. FAOSTAT. Food and Agricultre Organization of the United Nations Statistical; 2007.
6. Ahmed MMA, Anjum MA, Rabbani, Hassan L. Characterization of indigenous *Pyrus* germplasm of Azad Jammu and Kashmir revealed by SDS-PAGE analysis. Afric J Biotech. 2009;8(22):6442-6452.
7. Agong SG, Schittenhelm S, Friedt W. Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* L.) germplasm. Plant Genetic Resources Newsletter. 2000;123:61-67.
8. Karihaloo BL, Xiao QZ. Accurate determination of the coefficients of elastic crack tip asymptotic field by a hybrid crack element with p-adaptivity. Engng Fract Mech. 2001;68:1609–1630.
9. Furdi Florina. Assessment of genetic diversity in a collection of local tomatoes by SDS-PAGE method. J Hort Fores Biotech. 2012;16(3):133-136.
10. Phundan S, Narayanam SS. Biometrical techniques in plant breeding. Kalyani Publishers, New Delhi. 2009;1:1-343.
11. Machado S, Bynum JED, Archer TL, Lascano RJ, Wilson RJ, Bordovsky JE, Segarra KB, Nesmith DM, Xu W. Spatial and temporal variability of corn grain yield. Site-specific relationships of biotic and abiotic factors. Precision Agric. 2004;2: 343–360.
12. Mellingers JS. Measures of genetic similarity and genetic distance studies in genetics. VII. Uni Tex Publ. 1972;27(13): 145-153.
13. Hair JF, Barry CB, Babin J, Anderson RE, Tatham RL. Multivariate data analysis. Pearson Education Inc and Dorling Kindersely Publishing Inc; 2006.
14. Mohammadi SA. Statistical methods in genetics. Paper Presented at the 6th International Conference of Statistics, Uni of Tarbiat Modares Iran. 2002;7:26-28.
15. Malik AU, Haji MA, Bukhsh A, Hussain I, Athar MA, Ali M. Effect of seed rates sown on different dates on wheat under agroecological renditions' of Dera Ghazi Khan. J. Animal and Plant Sci. 2009;19(3): 126-129.
16. Ibitoye DO, Akin-Idowu PE, Ademoyegun OT. Agronomic and lycopene evaluation in tomato (*Lycopersicon lycopersicum* Mill.) as a function of genotype. World J Agric Sci. 2009;5(S):892-895.
17. Fehmida A, Ahmad SD. Morphogenetic comparison of three tomato cultivars from Azad Jammu and Kashmir, Pakistan. Sarhad J Agric. 2007;23(2):313-318.
18. Shuaib M, Jamal M, Akbar H, Khan I, Khalid R. The evaluation of wheat by polyacrylamide gel electrophorosis. African Journal of Biotechnology. 2010;9(2):243-247.
19. Sneath PHA, Sokal RR. Numerical taxonomy the principles and practices of numerical classification. W. F and Co. Freeman, San Francisco. 1973;1(1):1-573.
20. Shuaib M, Alam Z, Zahir A, Waqar A, Taufiq A, Ikhtiar K. Characterization of wheat varieties by seed storage protein electrophoresis. Afr. J. Biotechnol. 2007;6: 497-500.
21. Luckwill LC. The genus Lycopersicon, an historical, biological, and taxonomical survey of the wild and cultivated tomatoes. Aberdeen Uni Stud. 1943;120:1–44.
22. Rep MHL, Deckker JH, Vossen AD, Boer PM, Houterman CG, Koster D, Cornelissen BJC. A tomato xylem sap protein represents a new family of small cysteine-rich proteins with structural similarity to lipid transfer proteins. Federation of European Biochemical Societies. 2003;82-86.

23. Ding CK, Wang CY, Gross KC, Smith DL. Reduction of chilling injury and transcript accumulation of heat shock proteins in tomato fruit by methyl jasmonate and methyl salicylate. Plant Science. 2001;161: 1153–1159.
24. Singh JK, Singh JP, Jain SK, Aradhana J, Joshi A. Studies on genetic variability and its importance in tomato (*Lycopersicon esculentum* Mill.). Prog Hort. 2002;34:77-79.
25. Sevik H. Variation in seedling morphology of Turkish fir (*Abies nordmanniana* subsp. *bornmulleriana* Mattf). African Journal of Biotechnology. 2012;11(23):6389-6395.
26. Sevik H, Yahyaoglu Z, Turna I. Determination of genetic variation between populations of *Abies nordmanniana* subsp. *bornmulleriana* Mattf according to some seed characteristics, genetic diversity in plants. 2012;12:231-248. InTech. ISBN 978-953-51-0185-7.
27. Şevik H, Çetin M, Kapucu Ö. Effect of Light on young structures of Turkish Fir (*Abies nordmanniana* subsp. *bornmulleriana*). Oxidation Communications. 2016;39(1-II): 485-492.
28. Sevik H. Structure of genetic diversity in the populations of Turkish fir *(Abies nordmanniana* subsp. *bornmülleriana* Mattf.). Karadeniz Technical University, Graduate School of Natural and Applied Sciences (Doctoral dissertation, Doctorate Thesis); 2010.
29. Sevik H, Cetin M, Belkayali N. The determination of characteristics factors of grafted natural varieties in landscaping: A case study of black pine clone. Oxidation Communications. 2016;39(3-II):2820–2831.
30. Yigit N, Sevik H, Cetin M, Gul L. Clonal variation in chemical wood characteristics in Hanönü (Kastamonu) Günlüburun Black Pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe) seed orchard. Journal of Sustainable Forestry. 2016;35(7):515-526.
31. Sevik H. The differences among populations in Scotch pine (*Pinus sylvestris* L.) seed stands of Western Black Sea region. M.Sc Thesis, Gazi University Inst. Sci. Technol. 2005;60.
32. Sevik H, Yahyaoglu Z, Turna I. Analysis of seed characteristics from 17 different populations in *Abies nordmanniana* subsp. *bornmülleriana* Mattf. Proceedings of the 10th International Christmas Tree Research & Extension Conference, 18-24 August, Vienna; 2011.
33. Sevik H, Topacoglu O. Variation and inheritance pattern in cone and seed characteristics of Scots pine (*Pinus sylvestris* L.) for evaluation of genetic diversity. Journal of Environmental Biology. 2015;36(5):1125-1130.
34. Yiğit N. Micromorphological studies on plants and their importance. Developments in Science and Engineering. Editors: Recep Efe, Lia Matchavariani, Abdulkadir Yaldir, Laszlo Levai. Sofia; 2016. ISBN 978-954-07-4137-6.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18474