Genetic characterization of 22 of Tomato (Lycopersicon esculentum L.) genotypes using SDS-PAGE method

Attyaf Jameel Thamir AL-Tamimi1, Nidhal Abdul Hussein Al–Badeiry2 , Sinan Qais Khayoon3

Abstract : This study was conducted for characterization of 22 tomato varieties based on total soluble seed storage protein using electrophoresis on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) .Sex varieties show distinctive fingerprint (Sanam, Helam, Oula, Kenanh, Shady lady, Carioca) and low polymorphism (46%) was observed. The overall analysis of the results reveal high level of homogeneity was detected. Reduction in the genetic diversity among modern tomato cultivars may be attributed to the recent trend towards breeding for similar plant and fruit characteristics.

Key words Tomato, Genetic diversity, SDS-PAGE , UPGMA.

1.Introduction

Tomato (*Lycopersicon esculentum* L.) is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticultural industry worldwide [1].For many years, cultivars with narrow morphological variation were evaluated using traditional field plot techniques. This technique is tedious and time consuming. Furthermore, the morphological characters do not give the correct determination, because they are unstable and influenced by environmental conditions,. The biochemical markers makes more exact identification possible. In addition, the biochemical techniques are rapid , accurate and dependable [2]. As seed storage proteins are largely independent on environmental fluctuation, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of plant cultivars [3,4]. The electrophoretic protein profiles and their high stability and independence of the ecological conditions were used as cultivar markers [5,6].Seed protein analysis are very useful for differentiating and characterizing tomato genotypes [7],genetic diversity [8] ,F1 hybrid purity testing [9],tolerance and toxicity of some nutrient ([10], water deficit [11],abiotic stress [12,13,14],biotic stress [15]. As variety development is an important part of the plant breeding and the identification of these varieties by different parameters plays an important role in seed industry and seed trade [7] .The aim of this work was assessment of genetic variability among some tomato varieties based on seed protein and overall results will allow an excellent view of germplasm of tomato, especially as a matter of great theoretical and practical importance, namely the genetic diversity of tomato.

2. Materials and Methods

Twenty two of tomato genotypes were used for characterization based on protein profiles **(Table1)**. Electrophoretic technique of SDS-PAGE of total soluble seed proteins was carried out by using 12.5 per cent gels according to the method of [16] with slight modifications. Five sprouted (3days old) seeds were grounded in centrifuge tube by using micro pestle and 200µl Tris HCl extraction buffer (25mM, pH

8.8) was added. The mixture was agitated thoroughly and kept at 8C° for overnight for protein extraction. Then the mixture was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. This protein extract was dissolved in an equal volume of working buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.025% bromophenol blue) and incubated at 60-70ºC for 10 minutes, cooled immediately for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used for loading on to the gel. A current of 1.5 mA per well with a voltage of 80 V was applied until the tracking dye crossed the stacking gel.Later the current was increased to 2 mA per well and voltage up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using coomaasie brilliant blue solution overnight and destained using a mixture of 227ml of methanol, 46ml of acetic acid and 227ml of distilled water until the bands were clearly visible[7].

Table 1 Genotypes of tomato used for varietal characterization

No.	Genotype	Origin / company
1	GSN	France/ Asgrow
2	Sanam	Holland/ DEBBANE
3	Helam	Germany/ Goldpack
4	OULA	China/ Western Seed
5	Kenanh	Holland/ Enza Zaden
6	DOUNA	Peru/ Royal Sluit
7	Shady lady	Holland/ Nunhems
8	DALAL	USA/Royalcrownseed
9	BUSHRA	Germany/ Goldpack
10	Warda	USA/ US Agriseed
11	Fotton	China/ ASGROW
12	Super regina	USA/ Genetex
13	Carioca	China / GreenCo
14	Special pack	Holland/ Popvriend
15	Mongal	China / GreenCo
16	Supermarimond	France/ Vilmorin
17	Super Queen	USA/ Modesto seed
18	Shahirah	Holland/ Semen
19	Tamara	Holland/ Semen
20	248 Hybrid	USA/ Modesto seed
21	Sereen	Holland/ Popvriend
22	Cherry toato	USA/ US Agriseed

3.Results and Disscssion

The electrophoretic pattern of total soluble seed protein produced 15 main bands range in their molecular weight (170KDa-11 KDa),228 amplified bands.Seven were monomorphic , seven polymorphic and one unique band **(Table 2)** and **(Figure 1,2)**. Cluster analysis (phylogenetic tree) by UPGMA (unweighted pair-group method of arithmetic means) resulted in a dendrogram revealed that 22 genotypes of tomato divided in two main genetic groups the first large cluster include 17 genotypes and second small cluster include five genotypes. Each main group in turne divided into two subgroups **(Figure 3).**

Intensive homogeneity among tomato genotypes using biochemical marker was earlier repoted [8],despite this fact, electrophoretic pattern of total soluble seed protein produce powerful tool in studying tomato response to biotc [15] and abiotic [12,14] stresses.

4.Conclusions

In this study can be observe an extensive homogeneity among varieties of tomatoes analyzed. The conclusion is that the seed protein polymorphism in the best-known varieties of this species in our country has been shown to be very low at this level. Therefore these varieties should be studied also at the level of DNA by PCR technique, using different DNA markers.

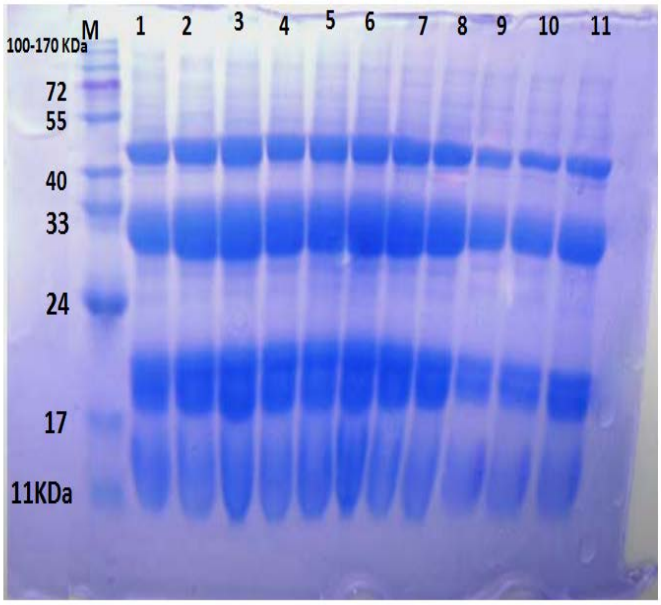

Figure 1 SDS-protein banding pattern of total soluble seed proteins of (1-11) tomato genotypes,M:Marker .1. GSN 2. Sanam 3. Helam 4. OULA 5. Kenanh 6. DOUNA 7. Shady lady 8. DALAL 9. BUSHRA 10. Warda 11. Fotton

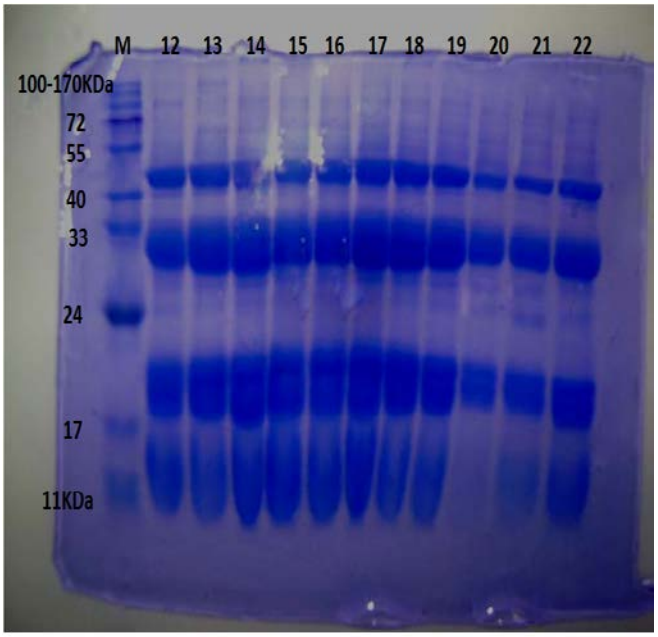

Figure 2 SDS-protein banding pattern of total soluble seed proteins of (12-22) tomato genotypes ,M:Marker.12. Super regina 13. Carioca 14. Special pack 15. Mongal 16. Supermarimond 17. Super Queen 18. Shahirah 19. Tamara 20. 248 Hybrid 21. Sereen 22. Cherry toato

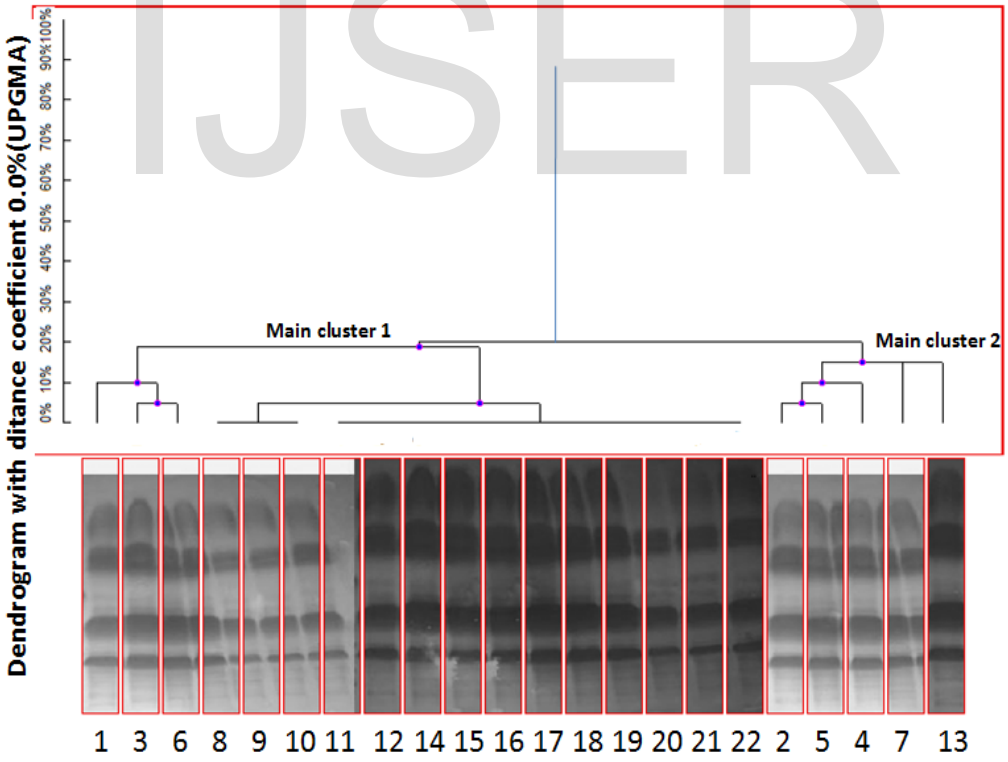

Figure 3 UPGMA dendrogram indicating the genetic relationships among 22 tomato genotypes based on total soluble seed protein

Table 2: Total seed protein patterns result from SDS electrophoresis of 22 Tomato genotypes : the presence of band (+), or absence (-) and fingerprinted genotypes (2- Sanam 3- Helam 4- Oula 5- Kenanh 7- Shady lady 13- Carioca)

No.of band	Tomato genotypes																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
2	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	-	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fingerprinted genotypes																						

5. References

[1] X. F. Wang, R. Knoblauch, and N. Leist.Varietal discrimination of tomato (*Lycopersicon esculentum* L.) by ultrathin-layer isoelectric focusing of seed protein.Seed Sci. Technol. 28(2005)521-526

[2] R. H.Sammour., Cultivars identification based on biochemical markers RRBS,8(9) (2014)347-358.

[3] R.H.Sammour.Plant Breeding,104(1989). 196-201,cited from R. H.Sammour.(2014), Cultivars identification based on biochemical markers RRBS,8(9):347-358.

[4] R.H.Sammour , A.E.Z.Mustafa, S.Badr ,W.Tahr;.Acta Agric.Slovenica, 88 (2007)33-43.cited in R. H.Sammour .Cultivars identification based on biochemical markers RRBS,8(9) .(2014) 347-358.

[5] R.H.Sammour , A.E.Z.Mustafa, S.Badr , W.Tahr;Acta.Bot.Croat., 66, 1-13 (2007). Cited from R. H.Sammour.Cultivars identification based on biochemical markers RRBS,8(9) .(2014), 347-358.

[6] R.S.Sammour , S.A.Radwan, M.Mira; Research and Review of Bioscience, 6, 351-360 (2012).cited in R. H.Sammour.Cultivars identification based on biochemical markers RRBS,8(9) .(2014), 347-358.

[7] K., Vishwanath, K. P. R., Prasanna, H.M.Pallvi,S.Rajendra Prasad , P J Ramegowda ,Devaraju , T. V Anantharayanan. Identification of Tomato (*Lycopersicon esculentum*) Varieties through Total Soluble Seed Proteins ,Research Journal

of Agricultural Sciences, 2(1)(2011) 08-12.

[8] F. Florina). Assessment of genetic diversity in a collection of local tomatoes by SDS-PAGE method Journal of Horticulture, Forestry and Biotechnology ,16(3) (2012 133- 136

[9] M.,Markova ,T.S. Stoilova. Biochemical Markers for Genetic Identification of Tomato (*Lycopersicon esculentum* Mill.) Soil Science Agrochemistry and Ecology, Sofia, XXXVIII, (2003) 1, 39-44.

[10] S. A.Abo-Hamad , S.S. El-Feky. Effect of Boron on Growth and Some Physiological Activities of Tomato Plant Life Science Journal;11(7) .(2014)403-408).

[11] R. A. Parchin A , M. Shaban. Effect of water deficit on total protein and protein profile in wheat (*Triticum aestivum* L.) genotypes International journal of Advanced Biological and Biomedical Research 2(2) (2014): 328-331.

[12] F. Aminia , A. A. Ehsanpour , Q. T. Hoang , J. Sh. Shin. Protein Pattern Changes in Tomato under In Vitro Salt Stress , Russian Journal of Plant Physiology, , 54(4) (2007) 464–471.

[13] Y. Yang , J.Guo.Characterization of LeCOP1 gene in *Lycopersicon esculentum* treated with various abiotic and oxidative stresses. African Journal of Biotechnology 11(99) (2012). 16478-16485.

[14] A. Manaa , H. Mimouni , S. Wasti , E. Gharbi , S. Aschi-Smiti M.,Faurobert , H. B. Ahmed. Comparative proteomic analysis of tomato (*Solanum lycopersicum*) leaves under salinity stress. plant omic journal, 6(4) (2013),268-277.

[15] İ. Berber , H. Onlu. The Levels Of Nitrite and Nitrate Profile and Protein Profils in Tomato Plants infected with *Pseudomonas syringae* ,J. Bot., 44(5) (2012)1521-1526.

[16] U. K.Lammeli .Cleavage of structural protein during the assembly of the head of BacteriophageT4.Nature.,227(1) (1970).680-685.