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ELECTROPHORETIC PROTEIN PROFILING OF DIVERSE TOMATO GERMPLASM CONTAINING UNIQUE GENES

SHIRIN AKHTAR[1]*, ABHISHEK NAIK[2], SUBHAMOY SIKDER[3], PRIYANKA BISWAS[2], JAYANTA TARAFDAR[4] AND PRANAB HAZRA[2]

[1]Department of Horticulture (Vegetable and Floriculture),
Bihar Agricultural University, Sabour - 813 210, Bihar, INDIA
[2]Department of Vegetable Crops,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur - 741 252, West Bengal, INDIA
[3]Department of Vegetable and Spice Crops,
Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal - 736 165, INDIA
[4]Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal - 741 252, INDIA
e-mail: shirin.0410@gmail.com

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***Corresponding author**

ABSTRACT

Total soluble seed storage proteins profiles of 12 tomato genotypes, including breeding lines containing four specific genes, *dg*, *hp-1*, *og*^{c} and *rin*, two exotic breeding lines and varieties each, two indigenously bred varieties and one local cultivar were analyzed by SDS-PAGE on 12.5% gels. Polymorphism of about 34.41% was obtained from seed protein profiling and 85.45% of the pairwise comparisons of the 12 genotypes showed at least one different protein band. The seed protein weight of the tomato genotypes under study ranged between 98KD to $<$14.3KD and relative mobility ranged between 0.025 and 0.787. Polymorphism in protein banding appeared in all the molecular weight regions, but maximum polymorphism occurred in comparatively lower molecular weight region (20.1 to 29 KD) and hence, this region is useful in identification of tomato genotypes. Dendrogram based on electro phoretic data clustered the 12 genotypes in three major clusters and four sub-clusters under Cluster I. The clustering pattern depicted unique genotypic features of BCT-115(*dg*), BCT-119(*hp*), Berika(high lycopene) and BCT-111*rin*(*rin*). BCT-115 was the most diverse genotype with one unique band. SDS-Protein profiling emerged important for genetic analysis and it indicated a considerable amount of genetic diversity among the tomato germplasm employed in the present investigation.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown in both temperate and tropical regions of the world with an estimated global production of about 163.03 million metric tonnes (FAOSTAT, 2015). Variety development is an integral part of the plant breeding and the identification of these varieties by different parameters plays an important role in seed industry and seed trade. However, with the increase in the number of varieties of each crop, it is difficult to distinguish the varieties on the basis of morphological characters alone. This has led to the development of the new stable parameters such as use of their genetic material (nucleic acids and proteins) as a tool for varietal identification. Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular trait. Therefore, the advent of the electrophoresis as an analytical tool provides indirect methods for genome probing by exposing structural variations of enzymes or other protein genome (Cooke 1984, Gilliland 1989). The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.*, 2001). Its banding pattern is very stable and has been widely suggested that such banding patterns could be important supplemental method for cultivars identification (Tanksley and Jones 1981, Thanh and Hirata 2002). Specific proteins and enzymes as markers have application in selection of parental material for hybridization, cultivar purity testing and explanation of phylogeny and taxonomy relations among different genus and species, F_1 hybrid purity testing etc. (Markova and Stoilova, 2003). Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding. These genetic markers are widely used as tools for the identification and estimation of the quantitative traits in plant resources, resistant to disease and environmental stress conditions, and other desirable agronomic traits (Zlokolica *et al.,* 1997).

The objective of the study was to detect differences in seed protein profiles of 12 tomato genotypes and possibility for their use in identification of different tomato lines and determination of their diversity.

MATERIALS AND METHODS

Twelve tomato genotypes, including breeding lines containing four specific genes, *dg, hp-1, og*^{c} and *rin*, two exotic breeding lines and varieties, each, two indigenously bred varieties and one local cultivar were used for the study. Basic charac-

ters and source of 12 genotypes of tomato used for characterization based on seed protein profiles have been presented in Table 1.

Electrophoretic technique of total soluble seed proteins

SDS-PAGE of total soluble seed proteins was carried out in the Department of Vegetable Crops, Bidhan Chandra Krishi Viswa vidyalaya by using 12.5% gels as per Laemmli (1970).

Seed protein was extracted using procedure described by Doonan (1996). 50mg of dried seeds of each genotype was homogenized using Tris HCl extraction buffer (25mM, *p*H 8.8). The homogenate was agitated thoroughly and kept at 8°C for overnight for protein extraction followed by centrifuging at 10,000 rpm for 15 minutes and the supernatant was collected. This protein extract was dissolved in an equal volume of working buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.025% bromophenol blue) and incubated at 60-70°C 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 a vertical SDS-polyacrilamide gel. Calibration kits (mixture of six characterized proteins: 97.4 kDa, 66 kDa, 43 kDa, 29 kDa, 20.1 kDa and 14.3 kDa) of GeNei™ manufactured by Merck (Catalogue No. 623110275001730) were used for analyzing the obtained electro phoreograms. The gel was run at a voltage of 80 V until the tracking dye crossed the stacking gel and then increased up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. The gel was stained using Coomassie 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.

Obtained electrophoreograms were analysed to calculate the Jaccard's coefficient of genetic similarity matrix using the software, SPSS version 11.0. Cluster analysis was performed to produce a dendrogram using unweight pair-group method with arithmetical average (UPGMA).

RESULTS

Identification of the varieties and breeding lines

The 12 genotypes under the present study differed in the number of bands, their relative mobility and intensity (Table 2, Figure 1). The proteins separated on 12.5 per cent acrylamide gel could be distinguished and grouped based on the standard marker (14.3 to 97.4 kD) (Fig. 1). By using SDS-PAGE, the total soluble seed protein could be fractionated into maximum 26 bands and minimum 17 bands, which showed heterogeneity among different genotypes. Polymorphism of around 34.61% was observed. 85.45% of the pairwise comparisons of the 12 genotypes studied showed atleast one different protein band.

BCT-115 possessing *dg* gene in it exhibited maximum number of bands (26) followed by three isogenic lines (Alisa Craig, Alisa Craig *hp-1* and Alisa Craig og^c) along with along with the indigenously bred variety BCT- 53 exhibiting 25 bands. The exotic variety of Europe (Berika) showed 24 bands, one indigenous cultivar, Pathar kutchi along with an early blight resistant variety FEB-2 and heat tolerant breeding line CLN B exhibited 23 bands. The line BCT-119 possessing *hp-1* gene exhibited 22 bands. The other heat tolerant breeding line CLN R exhibited the least number of 18 bands which is closely followed by 19 bands exhibited by the breeding line BCT 111*rin* carrying *rin* gene in it. The genotypes used in this study could also be differentiated by their banding intensity and relative mobility. When compared, some genotype had its unique profile which was different from other. Entire protein banding profile was divided in to six regions based on its decreasing molecular weight by comparing with standard protein marker (Fig. 1). The seed protein weight of tomato ranged between 98 kDa to < 14.3 kDa and relative mobility ranged between 0.025 and 0.787. In Region A (> 97.4 kDa, Phosphorylase b) all the genotypes showed two bands. Region B (66.0 to 97.4 kDa, Bovine serum albumin) showed two bands in 9 genotypes but 3 genotypes showed unique profiles, *viz*., CLN R with one missing band and BCT-119 (containing *hp-1* gene) and BCT-111*rin* (containing *rin* gene) with two missing bands. In Region C (43.0 to 66.0 kDa, Ovalbumin) 5 bands appeared in 10 genotypes but one each was missing in two genotypes CLN R and BCT-111*rin* (containing *rin* gene). In Region D (29.0 to 43.0 kDa, Carbonic anhydrase), 4 bands

Table 1: Sources and salient features of diverse tomato genotypes under study

Genotype(Variety/line)	Specific character or gene in it	Place of collection
Berika	Line bred variety having high lycopene variety	Institute of Physiology and Genetics, Bulgarian academy of Science, Sofia, Bulgaria
FEB-2	Line bred variety resistant to early blight disease	I.A.R.I., New Delhi, India
BCT-115 *dg*	High lycopne containing line possessing *dg* gene	United States Department of Agriculture, USA.
BCT-119	High lycopne containing line possessing *hp-1* gene	United States Department of Agriculture, USA
CLN B	Heat tolerant line low in carotenoid pigments	AVRDC, Taiwan
CLN R	Heat tolerant line low in carotenoid pigments	AVRDC, Taiwan
BCT-53	Line bred variety	Dept. of Vegetable crops, B.C.K.V., West Bengal, India
BCT-111*rin*	Line possessing ripening inhibitor *rin* gene	Haryana Agricultural University, Hisar, India
Alisa Craig	Old variety of Europe	Institute of Physiology and Genetics, Bulgarian academy of Science, Sofia, Bulgaria
Alisa Craig *hp-1*	An isogenic line of Alisa Craig with *hp-1* gene	Institute of Physiology and Genetics, Bulgarian academy of Science, Sofia, Bulgaria
Alisa Craig og^c	An isogenic line of Alisa Craig with og^cgene	Institute of Physiology and Genetics, Bulgarian academy of Science, Sofia, Bulgaria
Patharkutchi	Local adaptable cultivar medium in lycopene	Dept. of Vegetable crops, B.C.K.V., West Bengal, India pigment and high in acidity

Table 2: Intensity and relative mobility of total soluble seed proteins of tomato cultivars

Band No.	Relative mobility value	A	B	C	D	E	F	G	H	I	J	K	L
1	0.025	+	+	+	+	+	+	+	+	+	+	+	+
2	0.042	+	+	+	+	+	+	+	+	+	+	+	+
3	0.058	+	+	+	+	+	+	+	-	-	+	+	+
4	0.091	+	+	+	+	-	+	+	-	-	+	+	+
5	0.114	+	+	+	+	-	+	+	+	-	+	+	+
6	0.157	+	+	+	+	+	+	+	+	+	+	+	+
7	0.178	+	+	+	+	+	+	+	+	+	+	+	+
8	0.198	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +	+	+ +	+ +	+ +
9	0.218	+	+	+	+	+	+	+	+	+	+	+	+
10	0.234	+	+	+	+	-	+	+	+	-	+	+	+
11	0.272	+	+	+	+	+	+	+	+	+	+	+	+
12	0.315	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
13	0.345	+	+	+	+	+	+	+	+	+	+	+	+
14	0.363	-	+	+	+	-	+	+	+	-	+	+	+
15	0.391	+	+	+	+	+	+	+	+	+	+	+	+
16	0.419	+	-	+	-	-	-	+	+	-	+	+	+
17	0.434	+	-	+	-	-	-	+	+	-	+	+	+
18	0.459	+	+	+	+	-	+	+	+	-	+	+	+
19	0.487	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +	+	+ +	+ +	+ +
20	0.51	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +	+	+ +	+ +	+ +
21	0.53	+ +	+ +	+ +	+	+ +	+ +	+ +	+ +	+	+ +	+ +	+ +
22	0.563	+	+	+	+	+	+	+	+	+	+	+	+
23	0.584	-	-	+	-	-	-	-	-	-	-	-	-
24	0.612	+	+	+	+	+	+	+	+	+	+	+	+
25	0.711	+	+	+	+	+	+	+	+	+	+	+	+
26	0.787	+	+	+	+	+	+	+	+	+	+	+	+

A = Berika, B = FEB-2, C = BCT-115, D = CLN B, E = CLN R, F = Patharkutchi, G = BCT-53, H = BCT-119, I = BCT-111*rin*, J = Alisa Craig, K = Alisa Craig *hp*, L = Alisa Craig *og*^{c}

Table 3: Clustering pattern of the twelve diverse genotypes

Main Cluster	Sub-cluster	Genotype
I	a	Alisa Craig *hp*, Alisa Craig *og*^{c}, BCT-53, Alisa Craig
	b	Berika
	c	BCT-115
	d	BCT-119
II	a	CLN B, Patharkutchi, FEB-2
III	a	CLN R, BCT-111*rin*

appeared in 10 genotypes but one each was missing in two genotypes CLN R and BCT-111*rin* (containing *rin* gene). In Region E (20.1 to 29.0 kDa, Soybean Trypsin Inhibitor), 6 bands were observed in 6 genotypes while 1 band was found missing in Berika, 2 bands were found missing in 2 genotypes (FEB-2, CLN B and Patharkutchi) and 3 bands were missing in 2 genotypes (CLN R and BCT-111*rin*). In the region F (14.3-20.1 kDa, Lysozyme), 2 bands were found in 11genotypes while BCT-115 exhibited 3 bands, i.e., BCT-115 possessed one unique band.

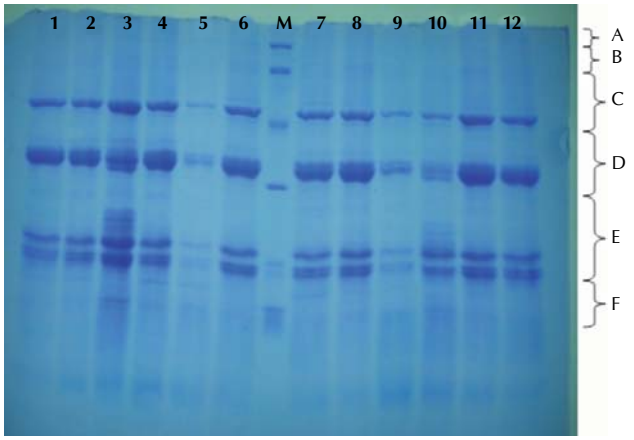
Phylogenetic diversity and relationship of the genotypes

The UPGMA method was used to calculate the similarity coefficient among the tomato genotypes, based on existence of the bands (presence or absence) and their average was used as an approximate value for recognizing groups of genotypes in dendogram (Fig. 2), which showed the same relationship among the genotypes

The cluster obtained from the dendogram (Table 3) showed that the studied tomato genotypes were grouped in 3 main clusters. Cluster I was subdivided into four sub-clusters, sub-cluster a comprising of 4 genotypes (Alisa Craig, Alisa Craig *hp-1* and Alisa Craig *og*^{c}, BCT- 53), while single genotype was grouped under sub-cluster b (BCT -115 possessing *dg* gene in it), sub-cluster c (BCT-119 possessing *hp-1* gene) and sub-cluster d (Berika, the variety of Europe) consisting of single genotypes. Cluster II consisted of 3 genotypes (FEB-2, CLN B and Patharkutchi) and Cluster IV had 2 genotypes (CLN R and BCT-111*rin* with *rin* gene in it).

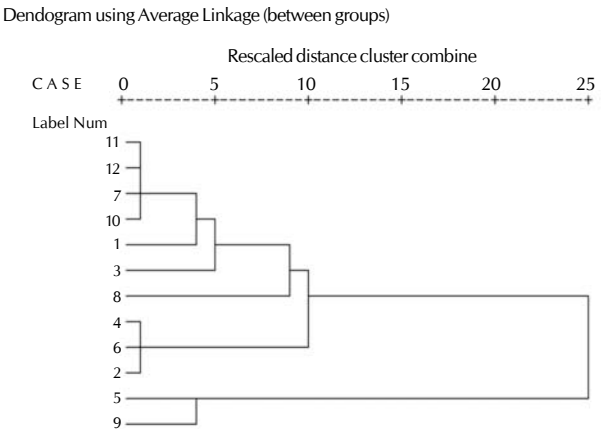
DISCUSSION

Many earlier studies showed protein electrophoresis as powerful tool to identify varieties of several crops like tomato (Chakrabarti *et al.*, 1992a; Wang *et al.*, 2000 ; Miskoska-Milevska *et al.* 2008; Chakrabarti *et al.*, 2010), eggplant (Mennella *et al.*, 1999), pepper (Lucchese *et al.*, 1999), pea (Drzewiecki, 1990) faba bean (Mudzana *et al.*, 1995), melon (Bonfitto *et al.*, 1999), maize, capsicum and rice in melon (Yan and Wand, 2003), barley, oat, wheat, peas and turnip (Ahokas, 2002), *Brassica rapa* (Rahman *et al.*, 2004), *Brassica juncea* L. (Rani and Rathore, 2006) and cucurbits (Dudwadkar *et al.*, 2015). Electrophoreograms and denzitograms of total, soluble and non-soluble proteins of different samples showed quantitative and qualitative differences in the protein profiles of tomato seeds in previous studies of Miskoska-Milevska *et al.* (2008) and Hamid *et al.* (2014). Its banding pattern is very stable which advocated for cultivars identification purpose in crops. It has been widely suggested that such banding patterns



1: Berika, 2: FEB-2, 3: BCT-115, 4: CLN B, 5: CLNR, 6: Patharkutchi, M: Marker, 7: BCT-53, 8: BCT-119, 9: BCT-111*rin*, 10: Alisa Craig, 11: Alisa Craig *hp*, 12: Alisa Craig *og*^{c}

Figure 1: Electrophoretic protein banding pattern of twelve diverse tomato genotypes



1: Berika, 2: FEB-2, 3: BCT-115, 4: CLN B, 5: CLNR, 6: Patharkutchi, M: Marker, 7: BCT-53, 8: BCT-119, 9: BCT-111*rin*, 10: Alisa Craig, 11: Alisa Craig *hp*, 12: Alisa Craig *og*^{c}

Figure 2: Dendrogram showing relationship among the genotypes

could be important supplemental method for cultivars identification (Tanksley and Jones, 1981 and Thanh and Hirata, 2002). Similar studies have been conducted by Abd El-Hady *et al*. (2010) who assessed phylogenetic diversity and relationships in eight tomato varieties by electro phoretic protein polymorphism. Endosperm seed protein analysis has been used for cultivar identification in complementation with morphological traits recorded in the field and alkali-soluble proteins were useful in differentiating ecotypes and cultivars while the other proteic fractions only showed a weak polymorphism (Mennella *et al.,* 2001). The high stability of protein profile has made protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz, 1979).

Electrophoretic protein profiling by SDS-PAGE is used for fractioning total soluble seed protein into different number of bands, varying in their relative mobility and intensity, which marked the heterogeneity among different genotypes. The proteins separated on the acrylamide gel could be distinguished and grouped based on the standard marker. The dissimilarity in the banding pattern showed the polymorphism thus depicting the unique character of the genotype under study. In this study the total soluble seed protein could be fractionated into maximum 26 bands and minimum 17 bands. Polymorphism of around 34.61% was observed. Weak polymorphism was obtained earlier by Mennella *et al*. (2001), Miskoska-Milevska *et al*. (2008) and Rodriguez *et al*. (2008). Dendrogram 85.45% of the pairwise comparisons of the 12 genotypes studied showed at least one different protein band clearly showing the diversity among the genotypes under study. Similar observations were recorded by Wang *et al*. (2000) who also recorded 87% of pairwise comparisons of the 11 varieties tested showing at least one different protein band.

The diversity of the genotypes was evident from their unique profile. Entire protein banding profile was divided in to six regions based on its decreasing molecular weight by comparing with standard protein marker. The banding pattern showed no polymorphism in the Region A (> 97.4 kDa, Phosphorylase b) where all the genotypes showed two bands. Region B (66.0 to 97.4 kDa, Bovine serum albumin) showed two bands in 9 genotypes but 3 genotypes showed unique profiles, *viz*., CLN R with one missing band and BCT-119 (containing *hp-1* gene) and BCT-111*rin* (containing *rin* gene) with two missing bands. In Region C (43.0 to 66.0 kDa, Ovalbumin) 5 bands appeared in 10 genotypes but one each was missing in two genotypes CLN R and BCT-111*rin* (containing *rin* gene). In Region D (29.0 to 43.0 kDa, Carbonic anhydrase), 4 bands appeared in 10 genotypes but one each was missing in two genotypes CLN R and BCT-111*rin* (containing *rin* gene). In Region E (20.1 to 29.0 kDa, Soybean Trypsin Inhibitor), 6 bands were observed in 6 genotypes while 1 band was found missing in Berika, 2 bands were found missing in 2 genotypes (FEB-2, CLN B and Patharkutchi) and 3 bands were missing in 2 genotypes (CLN R and BCT-111*rin*). In the region F (14.3-20.1 kDa, Lysozyme), 2 bands were found in 11genotypes while BCT-115 exhibited 3 bands, i.e., BCT-115 possessed one unique band. It appeared that polymorphism in protein banding appeared in all the molecular weight regions, but maximum polymorphism occurred in comparatively lower molecular weight region (20.1 to 29 kDa) hence, this region could be useful in identification of tomato genotypes. In the study of Fehmida and Ahmad (2007) comparisons of the total seed proteins based on SDS-PAGE profile did not indicate variability among the major bands. Hamid *et al*. (2014) reported very less variability among the bands while studying the protein profile of tomato germplasm that included hybrids. However, some minor bands were found to be variable in terms of the distance travelled in the gel.

The clustering pattern depicted unique genotypic features of some genotypes like BCT-115*dg* (possessing *dg* gene), BCT-119 (possessing *hp-1* gene), Berika (high lycopene containing variety of Europe) and BCT-111*rin* (possessing *rin* gene). The European variety Alisa Craig and its isogenic lines Alisa Craig *hp-1* and Alisa Craig *og*^{c} introgressed with *hp-1* and *og*^{c} genes respectively exhibited 100% homology. The indigenous line bred variety BCT-53 also showed 100% homology with these

three genotypes. The breeding lines BCT-115 containing *dg* gene and BCT-119 containing *hp-1* gene and Berika, the European variety with high lycopene content are single members of the sub-clusters of Cluster I. All the members of the Cluster I may be linked by the trait high pigment content. The clustering of the genotypes CLN R, a heat tolerant breeding line with low lycopene content and BCT-111 *rin* possessing ripening inhibitor gene is surprising, but fruit firmness and low lycopene content in both genotypes may be responsible for the linkage of the two genotypes. The genotypic relationship between the members of the third cluster, i.e., FEB-2, an early blight resistant variety, CLN B, a heat tolerant breeding line and Patharkutchi, a highly acceptable local cultivar of West Bengal is difficult to interpret, but high acidity and low pigment content of all three genotypes may have role in the close linkage. Cluster analysis in tomato carried out by Meena and Bahadur (2013) has been found to depict similar results.

From this study it could be concluded that alkaline protein electrophoresis could differentiate between the tomato varieties and produce some specific bands that may be used to distinguish genotypes from each other. Further analysis of these specific variations could be done to assess the protein polymorphisms between different genotypes of tomato and clarify the genetic nature of polymorphic bands. However, weak polymorphism in SDS-PAGE banding patterns of was reported in the present investigation. This clearly indicated that morphological and biochemical methods of genotype characterization of tomato are not alternative methods, but they are complementary to each other.

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