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EVALUATION OF GENETIC DIVERSITY IN DIFFERENT GENOTYPES OF *ERUCA SATIVA* FROM PAKISTAN BY SDS-PAGE ANALYSIS

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Abstract

The *Eruca sativa* (Taramira) germplasm, comprising 102 accessions was evaluated for total seed storage proteins via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The accessions were collected from different ecological areas of Pakistan. Total seed storage proteins were electrophoretically separated on 12.5 to 15.0% polyacrylamide gels. A total of 17 protein bands were detected, of which 6 (35%) were monomorphic and 11 (65%) were polymorphic, with molecular weight extending from 15 to 220 kDa. Dice coefficients among accessions ranged from 0.60 to 1.00. The dendrogram based on dissimilarity matrix using unweighted pair group method with arithmetic averages (UPGMA) divided all the accessions into 4 main groups i.e., 1, 2, 3 and 4 comprising 2, 15, 2 and 83 genotypes, respectively. Although a low level of genetic diversity was observed among given germplasm but the presence/absence and different protein banding pattern showed a considerable level of variability among different Taramira accessions. The variations revealed in this study should be exploited for the future breeding potential of Taramira germplasm by using other advanced molecular techniques including 2-D gel electrophoresis. No studies have yet been conducted in Pakistan on the genetic assessment of *Eruca sativa* germplasm based on total seed protein. This evaluation will significantly help for identification and differentiation of Taramira germplasm and for best utilization in Taramira varietal improvement program in Pakistan.

Introduction

Taramira (*Eruca sativa*) is an important leafy vegetable and an oilseed crop that belongs to family Brassicaceae. It is thought to be native of North Africa and South Europe, and is cultivated in other countries including Canada, China, Germany, France, Poland and Sweden, and to an extent in India and Pakistan. *Eruca sativa* Mill (Taramira), *Eruca vesicaria* and *Eruca pinnatifida* are three important species of genus *Eruca* and mostly found in Mediterranean countries, Central Asia and Europe (Warwick *et al*., 2007). It is also cultivated widely in western and central Asia for oil, termed 'jamba-oil' in India (Al-Shehbaz, 1985; Yaniv *et al*., 1998; Specht & Diederichsen, 2001). Taramira original husbandry dates back to ancient Greeks and Romans. It is mostly grown on marginal lands with reduced and poor soil fertility. Taramira mostly preferred over other relative species owing to its compliance to harsh environmental surroundings and stress nature (Gupta *et al*., 1998). That is why it is particularly fit for the regions having scanty or no irrigation facilities. The seed oil content (22 to 41%) of Taramira is rich in erucic acid (Al-Shehbaz, 1985; Yadava *et al*., 1998; Mandal *et al*., 2002), which marks Taramira species a possible future spring of industrial oil (Yaniv *et al*., 1998). The oil extracted from Taramira seed contains substantial amounts of glucoerucin and antioxidant activity (Barillari *et al*., 2005). The seed oil of this important oil crop can be utilized in lubricant, lamp oil, human nutrition and for many different cosmetic and medicinal purposes (Al-Shehbaz, 1985; Yaniv *et al*., 1998). Taramira is a vital oilseed crop, it was given negligible weight and so, the yielding ability is very much limited (Gupta *et al*., 1998). Inadequate material is offered on the nature of morphological and genetic diversity, and relationship of *E. sativa* genotypes.

Genetic rise of the crop and the growth of a species need the simplicity of access of genetic diversity. Discovery of replica, organization of central set of a definite population and the choice of range of parents for the breeding needs are directly linked to the genetic variability. Genetic diversity assessments of different crops have been studied by different researchers. Akbar *et al*., (2011) studied genetic diversity of 20 *Sesamum indicum* L. accessions at DNA level by means of random amplified polymorphic DNA (RAPD) analysis. Similarly Shinwari *et al*., (2012) examined genetic diversity of 100 *Eruca sativa* genotypes collected from different ecological area of Pakistan were evaluated for twenty quantitative and 5 qualitative traits. Akbar *et al*., (2011) also investigated genetic diversity of sesame germplasm using sixteen quantitative and qualitative characters. Various techniques have been considered to assess diversity by means of biochemical, morphological and physiological categorization (Greene *et al*., 2004). The use of biochemical markers has acknowledged. Among the biochemical techniques, SDS-PAGE is commonly used due to its easiness and usefulness (Khan *et al*., 2013; Sultan *et al*., 2013; Siddiqui *et al*., 2010). Akbar *et al*., (2012) studied genetic diversity of *Sesamum indicum* via total seed protein using SDS-PAGE technique and got satisfactory results. The objective of present study was to examine genetic diversity of 102 Taramira accessions based on SDS-PAGE analysis, collected from diverse ecological regions of Pakistan. Similar studies were conducted by Zada *et al*., (2013) and Shah *et al*., (2011).

Materials and Methods

Plant material and protein extraction: One hundred and two Taramira accessions were obtained from PGRP Gene-bank, Institute of Agri-Biotechnology & Genetic Resources (IABGR), NARC, Islamabad. This material was collected from different ecologies of Pakistan (Table

1). For the proteins extraction, seeds were powder through mortar and pestle. About 0.1 gram seed powder was put into 1.5ml micro-tube and protein extraction buffer (400µl) was added to it. The extraction buffer composed of Tris-HCl 0.5M (pH 8.0), SDS 0.2%, Urea 5M and 2-mercaptoethanol 1%. Dye (Bromophenol blue) was added to display the movement of protein. Eventually samples were mixed carefully by vortexing and centrifugation at 15,000 rpm for 10 to 12 minutes at room temperature (RT), and kept at -4^{o}C till gel electrophoresis process.

Preparation of electrophoretic gel and electrophoresis: Total seed protein electrophoresis was carried out in twenty percent polyacrylamide slab gels in discontinuous buffer system according to Laemmli (1970) method. The separating gel solution contained acrylamide 20% and N.N-methylene-acrylamide 0.135% in 0.5M Tris-HCl buffer (pH 8.8) with SDS 0.27%. The gel was polymerized by adding10% APS (Ammonium per sulphate) and 15 microlitters TEMED (Tetramethylene-diamine). The staking gel solution comprised of acrylamide 30% and N.N-methylene-bis-acrylamide 0.8% in 0.25M Tris-HCL buffer (pH 6.8) having SDS 0.2. The electrode buffer was a mixture of Tris-glycine (9.0g Tris-HCl and 43.2g glycine per 3 liters buffer solution at pH 8.9) and SDS 3.0g. Ten to 12 microliters of protein sample was added into the wells. Electrophoresis was carried out at 90V for about 2.30 to 3 hours till blue marker reached at the bottom of gel. The molecular weights of separated protein bands were compared with standards protein ladder ranging from 10 to 220 KDa (Invitrogen). Later, the gels were stained with commassie blue solution from 40 to 60 minutes. Gels were then destained with destaining solution containing acetic acid (5%), methanol (20%) and distilled water in the ratio of 5:20:75 (v/v) for more than 2 hour (Fig. 1).

Table 1. List of *Eruca sativa* accessions used in present study.

No.	Accession	Collection area	No.	Accession	Collection area	No.	Accession	Collection area
1.	3709	-	35.	3675	Attock	69.	1759	Badin
2.	3710	-	36.	3676	Attock	70.	1760	D.G.Khan
3.	3711	-	37.	3677	Haripur	71.	1767	Khushab
4.	3712	-	38.	3678	Rawalpindi	72.	3644	Attock
5.	17381	Chakwal	39.	3679	Rawalpindi	73.	3645	Chakwal
6.	17382	Jhelum	40.	3680	Rawalpindi	74.	3646	Chakwal
7.	17385	Khushab	41.	3681	Chakwal	75.	3647	Chakwal
8.	17386	Bhakkar	42.	3682	Chakwal	76.	3648	Chakwal
9.	17387	Layyah	43.	3683	Chakwal	77.	3649	Bhakkar
10.	17388	D.G.Khan	44.	3684	Chakwal	78.	3650	Khushab
11.	17389	Lakki Marwat	45.	3685	Chakwal	79.	3651	Vehari
12.	17390	Attock	46.	3686	Chakwal	80.	3652	Bahawalpur
13.	17391	Attock	47.	3687	Chakwal	81.	3653	Bahawalpur
14.	17392	Attock	48.	3688	Chakwal	82.	3654	Rajanpur
15.	17393	Attock	49.	3689	Khushab	83.	3655	Rajanpur
16.	17394	Chakwal	50.	3690	Sargodha	84.	3656	Rajanpur
17.	17395	Chakwal	51.	3691	Sargodha	85.	3657	Rajanpur
18.	17396	Chakwal	52.	3692	Hafizabad	86.	3658	Rajanpur
19.	17397	Chakwal	53.	3693	Jhelum	87.	3659	Rajanpur
20.	17398	Chakwal	54.	3694	Jhelum	88.	3660	Rajanpur
21.	17399	Chakwal	55.	3695	Jhelum	89.	3661	D.G.Khan
22.	17400	Chakwal	56.	3696	Rawalpindi	90.	3662	D.G.Khan
23.	17402	Chakwal	57.	3697	Sargodha	91.	3663	D.G.Khan
24.	17403	Chakwal	58.	3698	Sargodha	92.	3664	D.G.Khan
25.	17405	Chakwal	59.	3699	T.T.Singh	93.	3665	D.G.Khan
26.	17406	Chakwal	60.	3700	Faisalabad	94.	3666	D.I.Khan
27.	17407	Mianwali	61.	3701	Okara	95.	3667	D.I.Khan
28.	17408	Chakwal	62.	3702	Sahiwal	96.	3668	D.I.Khan
29.	17409	Hangu	63.	3703	Pakpattan	97.	3669	D.I.Khan
30.	17410	Kohat	64.	3704	Kasur	98.	3670	D.I.Khan
31.	17411	Attock	65.	3705	Kasur	99.	3671	Lakki Marwat
32.	17412	D.I.Khan	66.	3706	Sheikhupura	100.	3672	Karak
33.	3673	Karak	67.	3707	Rawalpindi	101.	26187	Netherlands
34.	3674	Attock	68.	3708	Umarkot	102.	27460	AARI, Faisalabad

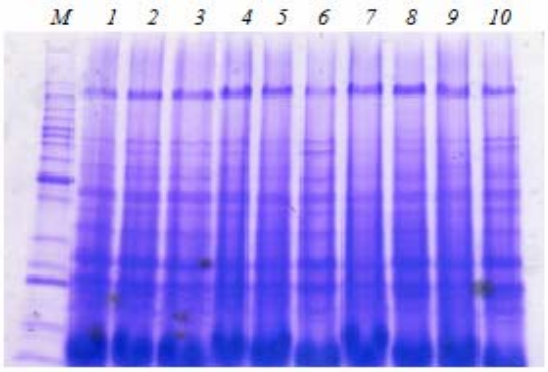

Fig. 1. Electrophoretic banding pattern produced by SDS-PAGE of total seed proteins of Taramira genotypes.

Data analysis: Based on presence / absence of total seed protein bands, similarity index was deliberated for all potential pairs of protein types. The score was 0 and 1 for absence and presence of protein bands, respectively. Similarity index (s) was designed for all conceivable pairs of protein type by means of the following formula (Sneath & Sokal, 1973):

$$S = w / (a + b – w)$$

where, s, w, a, b represent similarity index, number of bands of common mobility, number of bands of protein type 'a' and number of bands in protein type 'b', respectively. The similarity matrix thus engendered was renewed into a dissimilarity matrix (dissimilarity = 1 – similarity) and used to make dendrogram via un-weighted pair-group method with arithmetic averages (Sneath & Sokal, 1973). All the analyses were conducted by applying statistical package NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Results

A total of 17 protein polypeptide bands were observed among the 102 Taramira accessions assessed. Of these 17 bands, 6 (35%) were monomorphic and 11 (65%) were polymorphic. Size of the protein bands (compared with a standard Unstained Protein Molecular Weight Marker ranging from 10 to 220 kDa) fluctuated from 15 to 220 kDa. Four out of 17 bands i.e. 2, 7, 12 and 16 were common in all Taramira accessions, whereas band 6 was present in 13 out of 102 Taramira accessions and band 10 was missing in 20 accessions only. Accessions with minimum proteins bands were, 17394 (Chakwal) 3686 (Chakwal), 3667 (D.I. Khan) and 3668 (D.I. Khan), 3665 (D.G. Khan), 3684 (Chakwal) and 17382 (Jhelum). They have 8 to 10 protein bands only. While some accessions showed maximum protein bands such as, 27460 (D.G. Khan), 17400 (Chakwal) and 3656 (Rajanpur) showed maximum 16 bands, while accessions 26187 (D.G. Khan), 17409 (Hangu), 17410 (Kohat), 17381 (Chakwal) and 1759 (Badin) showed 15 polypeptide bands. Only clear scorable bands were considered and scored for statistical analysis. Minor protein bands showed less variation as compared to the major protein bands of the gel. Similarly variability in intensity was seen in numerous bands that presented the aggregate of protein peptides swelling up at a definite molecular weight.

The cluster diagram revealed 4 major Clusters i.e., '1', '2', '3' and '4' (Fig. 2). Cluster 1 and 3 were the smallest clusters having two accessions each. Cluster 4 was the largest cluster with maximum number of 84 accessions, Cluster 4 was further divided into 2 sub-clusters i.e., sub-cluster I and II with 73 and 10 accessions, respectively. The second largest cluster was cluster '2' with maximum 17 Taramira accessions. Cluster '2' was further divided into 2 sub-clusters i.e., sub-cluster I and II with 14 and 03 accessions each (Table 2). Similarity coefficients ranged from 0.60 to 1.00.

Discussion

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a handy tool for studying genetic assortment of crops in a short period of time (Sadia *et al*., 2009; Netra & Prasad, 2007). SDS-PAGE analysis can be easily utilized for numerous purposes, such as categorization of germplasm, biosystematics study, varietal certification, and determination of phylogenetic association between diverse species and generation of relevant information to balance estimation (Iqbal *et al*., 2005) beside other markers like RAPD (Jan *et al*., 2011; Pervaiz *et al*., 2010) or microsatellite markers (Rabbani *et al*., 2010). Inspite of being an important oilseed crop, Taramira is a neglected crop in Pakistan. Hence, it becomes extremely imperious to assess inter and intra specific genetic diversity of Taramira germplasm for varietal improvement to extend the germplasm base in the future breeding programs for the viable organization of the genetic resources in Pakistan. Cluster analysis provides useful information to recognize divergent parentages tied with the nearby genetic affiliation among numerous crop species for better manipulation of hybrid generation of widespread variability for crop upgrading (Maity *et al*., 2009). Accessions collected from D.G. Khan, D.I. Khan and Chakwal showed extremely wide range protein band variation i.e., some accessions exhibited minimum number of protein bands, while other accessions from the same area showed maximum number of protein bands. More collections of native landraces should be made from these areas as most types of variants were documented from these areas. It is obvious from the dendrogram that 'accession 3655 from Rajanpur' and 'accession 1759 from Badin' are most distinctly allied with each other hence, these two accessions should be considered in future for breeding programs to get higher level genetically variable variety of Taramira. Present biochemical assessment provides first information to document Taramira genotypes in Pakistan based on SDS-PAGE. Present research activity will be helpful to make a gene bank of genetic resources of diverse Taramira genotypes in Pakistan.

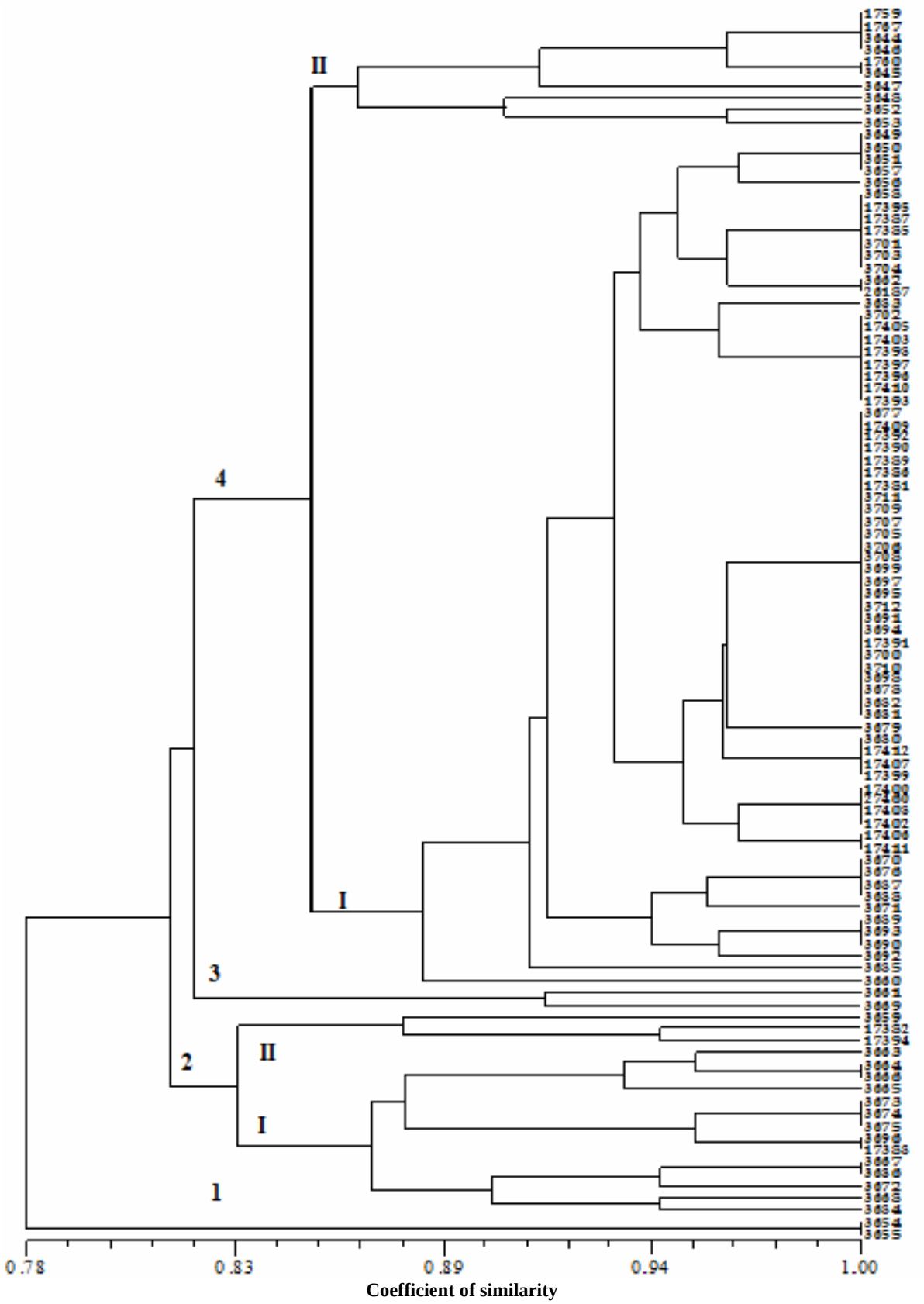

Fig. 2. Dendrogram presenting association among 102 Taramira accessions based on SDS-PAGE analysis.

Table 2. Grouping of 102 *Eruca sativa* genotypes based on cluster analysis using SDS-PAGE analysis.

Clusters	Sub-clusters	No. of genotypes	Genotypes
1	-	2	3654 & 3655
2	I	14	3663, 3664, 3665, 3666, 3667, 3668, 3672, 3673, 3674, 3675, 3686, 3696, 17384 & 17388
	II	03	3659, 17382 & 17396
3	-	02	3661 & 3669
4	I	73	3649, 3650, 3651, 3656, 3657, 3658, 3660, 3662, 3670, 3671, 3676, 3677, 3678, 3679, 3680, 3681, 3682, 3683, 3685, 3686, 3687, 3688, 3689, 3690, 3691, 3692, 3693, 3694, 3695, 3697, 3698, 3699, 3700, 3701, 3702, 3703, 3704, 3705, 3706, 3707, 3708, 3709, 3710, 3711, 3712, 17381, 17385, 17386, 17387, 17389, 17390, 17391, 17392, 17393, 17395, 17396, 17397, 17398, 17399, 17400, 17402, 17403, 17405, 17406, 17407, 17408, 17409, 17410, 17411, 17412, 26187 & 27460
	II	10	1759, 1760, 1767, 3644, 3645, 3646, 3647, 3648, 3652 & 3653

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