

GENETIC DIVERSITY IN RADISH GERMPLASM FOR MORPHOLOGICAL TRAITS AND SEED STORAGE PROTEINS

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Abstract

Genetic variation of forty-nine local and exotic radish genotypes including two checks was studied for morphological traits and seed storage protein electrophoresis using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) markers. A high variation in germplasm for root shape, root length, root colour (internal and external), flesh texture and root type was observed. Among these genotypes, the genetic variation was apparent for most of the characters like plant biomass, root weight, leaf length, root length and root diameter that indicated the potential for crop improvement in these traits through simple selection. Exotic germplasm exhibited higher variation for plant biomass, root weight and root length which could be utilized through breeding programme. Cluster analysis on the basis of genetic diversity for seven quantitative traits resulted into four clusters. No clustering was found on the basis of origin. Low level of variance was observed for SDS-PAGE electrophoresis that suggested acquisition of more germplasm. On the basis of high yield and crispy root texture some genotypes (10076, 10362, 10429, 10658, 10662 and 10667) were identified for further testing under wide range of agro-ecological conditions.

Introduction

Broad based plant genetic resources are imperative for sound and successful crop improvement programme. The diversity of cultivated plant species depends upon mutation and hybridization, range of dispersal and processes of cultivation and domestication (Yamane *et al.*, 2009; Heiser, 1988). Introduction of improved varieties and attempts at seed production utilizing such varieties as mother plants may lead to loss of the diversity of land races (Nisar & Ghafoor *et al.*, 2011; Mumtaz *et al.*, 2010; Pervaiz *et al.*, 2010). Radish (*Raphanus sativus*) has a short life cycle and possesses many genetic markers, male sterility and a multi-allelic incompatibility system due to which it is becoming useful in molecular genetics for experimentation (Crisp, 1995). Adequate description of the diversity of radish should result in much greater emphasis on both conserving and exploiting this variation (Crisp, 1995).

Radish is an important root vegetable and grown world-wide. It occupies an important position among vegetable crops in Pakistan. It was cultivated over an area of 10133 hectares producing 173806 tones (Anon., 2009). It is widely used as root vegetable, however, its immature young green pods are also used as a vegetable by a large population. Indigenous cultivars are mostly white having a triangular or cylindrical shape roots with more pungent taste than introduced types. Genetic variability of radish germplasm is not much investigated and only a few studies have been reported on phenotypic diversity (Nomura *et al.*, 1996). As compared to other crucifer species, the diversity of radish is not yet characterized at genetic and molecular levels despite its world-wide economic importance (Rabbani *et al.*, 1998; Rabbani *et al.*, 2010). In the present study we report the genetic variation in radish germplasm from Pakistan and Japan,

evaluated and characterized under field conditions for various qualitative and quantitative traits.

Materials and Methods

Germplasm evaluation for various morphological and agronomical traits was conducted in an augmented design under the field conditions of National Agricultural Research Centre, Islamabad. The germplasm (49 accessions) were obtained from National Genebank, Institute of Agri-Biotechnology and Genetic Resources (IABGR), NARC, Islamabad. Among these, 30 accessions were of Pakistan origin whereas 17 accessions were of Japanese origin. One row of two-meter length for each accession was planted with 20cm plant spacing and 75cm row spacing. Two check varieties (Minowase and Minochal, obtained from Horticulture Research Institute, NARC, Islamabad) were repeated after every 15 rows. Recommended cultural practices were followed throughout the crop season. Data were recorded for qualitative as well as quantitative traits. Qualitative characters were recorded on plant basis for leaf shape, leaf division margins, leaf division incision, leaf apex, leaf colour, leaf hairiness, root type, root shape, root shoulder, root base, green colour on shoulder, exterior and interior root colour, colour distribution within flesh and root flesh texture. Quantitative characters like plant biomass (g), number of leaves, leaf length (cm), leaf blade width (cm), root weight (g), root length (cm) and root diameter (cm) were recorded. Trait selection and measurement techniques were based on IBPGR descriptors for brassica and *Raphanus*, 1990. Root growth measurements were taken at the time of root harvest (marketable root stage). The averaged data were analyzed statistically, for means, standard deviation, frequency distribution and simple correlation coefficients.

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All the accessions were subjected to seed protein analysis through sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed proteins were extracted from single grain with 0.5ml of 0.05M Tris-HCl Buffer (pH 8.0), with the concentration of 0.2% SDS and 5 M urea. Extraction buffer 5 μ l of 2-mercaptoethanol was added to this sample and properly mixed. Seed proteins were analyzed through the slab type SDS-PAGE using 11.25% polyacrylamide gel at 100V for two and half hours. The gels were stained with Commassie Brilliant Blue and de-stained in 5% methanol and 20% acetic acid until the color of background disappeared. After de-staining, gels were dried and the data were recorded for repeatable and readable bands.

Data were analyzed for genetic diversity using computer programme STATISTICA for Windows. Ward's method was used to construct the phenogram and calculate Euclidean distances among the accessions as an estimate of the genetic distances. The means and standard

deviation for quantitative characters within each cluster were calculated to estimate the inter cluster variation.

Results and Discussion

Basic statistics for the quantitative traits presented in (Table 1) revealed high variance for number of leaves, leaf length and leaf blade width in local germplasm whereas exotic germplasm produced higher values for plant biomass, root length, root diameter and root weight. There seems to be a scope for further improvement in respective traits both in local and exotic germplasm. High variation for various traits could be used to establish groups in the present germplasm. A varying degree of variation was depicted for yield contributing traits both in local and exotics. However, for root weight, root length and root diameter exotic germplasm performed better. In the similar studies, variation in root fresh weight, root length and width have been reported by Sudan *et al.*, (1991).

Table 1. Basic Statistics for different quantitative traits in local and exotic radish germplasm.

Parameters	Origin	Mean+ stand dev.	Minimum	Maximum
Plant biomass (g)	Local	417.09 \pm 132.31	170.18	693.37
	Exotic	442.51 \pm 181.04	155.60	698.20
Leaves (No.)	Local	23.93 \pm 4.59	16.50	35.00
	Exotic	22.41 \pm 3.84	16.00	29.00
Leaf length (cm)	Local	39.52 \pm 10.10	14.90	54.70
	Exotic	34.41 \pm 4.17	25.60	41.28
Leaf diameter (cm)	Local	12.12 \pm 2.48	7.77	17.67
	Exotic	11.64 \pm 1.59	8.80	14.23
Root length (cm)	Local	24.53 \pm 6.80	13.10	40.15
	Exotic	24.04 \pm 10.14	9.00	44.10
Root diameter (cm)	Local	4.39 \pm 0.89	3.09	7.92
	Exotic	4.64 \pm 1.16	3.00	7.53
Root weight (g)	Local	235.09 \pm 89.16	108.80	450.54
	Exotic	280.70 \pm 133.14	81.10	513.55

For leaf blade width and root diameter low genetic variability was observed that restricts the scope of selection for these traits in the present germplasm collection, therefore, large scale testing of broad base germplasm need to be build up by making extensive local collections and obtaining germplasm from abroad to develop a sound breeding programme (Yousaf *et al.*, 2008; Mahmood *et al.*, 2010; Jan *et al.*, 2011; Yousaf *et al.*, 2011).

Morphological Characteristics

Qualitative traits: Various distinct groups for morphological traits are presented in (Table 2). High variation in root type, flesh texture, interior root colour, root shoulder and leaf division margin was observed. For these characters, selection for various genetic markers could be utilized by breeders. But for traits where low variability was observed, the accessions with distinct traits are suggested to be acquired or collected from the centers of maximum diversity.

Quantitative traits: The frequency distributions for various quantitative traits are presented in Fig. 1. A high genetic variance was observed for plant biomass, number of leaves, leaf length, root length and root weight whereas

for rest of plant traits a low genetic variation was observed. It is a good hope that genetic variation in present germplasm for root length and root weight could be a future potential source for radish improvement. Genetic variation in number of leaves and leaf length could be utilized for improvement in radish germplasm to be used as fodder or pod purposes.

Correlation studies: The correlation coefficients were computed for two sets of accessions separately among quantitative traits and presented in the (Table 3). Results revealed that plant biomass had a positive correlation with all characters. Number of leaves showed a positive association with root length, root diameter and root weight whereas leaf length and leaf blade width showed a positive trend with all parameters except with root length. Park *et al.*, (1989) also reported positive correlation of root length with root weight and number of leaves. There was a negative correlation of root length with root width. Root diameter had a positive association with root weight. Leaf length and leaf blade width showed a negative correlation with root length, which reflected a negative impact on root yield as root length and root weight, was positively correlated. However, this could be utilized for further improvement in these characteristics that are to be utilized for fodder or fruit purposes. For exotic germplasm

plant biomass, leaf length, root weight and leaf blade width had a positive correlation with all the characters. Root diameter had a strong association with all characters under study that indicated importance of simple selection for improved root size. Number of leaves and root length showed a positive association for all the parameters except root diameter in both cases. Negative correlation was found between root length and root diameter, which was also reflected in local germplasm. A strong association between root weight and rest of parameters were observed in local as well as exotic germplasm,

depicting a similar trend in both the cases. Park *et al.*, (1989) also reported positive correlation of root length with root weight and number of leaves. For character pairs where association was similar are suggested to be employed in a broad spectrum but in character pairs with different correlation magnitude, the germplasm is needed to be handled with care for improvement through simple selection. The undesirable negative correlations are suggested to be broken by mutation breeding or selective parent diallel.

Table 2. Frequency distribution of qualitative plant traits in radish.

Parameter	Frequency	Percent	Parameter	Frequency	Percent
Leaf shape			Green col. on shoulder		
Spathulate	116	97.48	No	86	72.27
Elliptic	1	0.84	Low	16	13.45
Obvate	2	1.68	Intermediate	15	12.61
Leaf division margin			High	2	1.68
Dentate	87	73.11	Root shoulder		
Crenate	22	18.49	Plane	113	94.96
Serrate	10	8.40	Convex	6	5.04
Leaf division incision			Root colour exterior		
Lyrate	112	94.12	White	104	87.39
Sinuate	7	5.88	Pink	2	1.68
Leaf apex			L/green	12	10.08
Round	68	57.14	Dark Green	1	0.84
Intermediate	51	42.86	Root type		
Leaf colour			Short (below 15cm)	6.00	5.04
Green	105	88.24	Intermediate(16-30cm)	77.00	64.71
Dark Green	5	4.20	Long (above 30 cm)	36.00	30.25
Light Green	9	7.56	Root colour interior		
Leaf hairiness			White	85	71.43
Intermediate	114	95.80	Dull white	10	8.40
Sparse	5	4.20	Greenish white	19	15.97
Root shape			Pink	4	3.36
Triangular	110	92.44	Pinkish white	1	0.84
Elliptic	4	3.36	Col. distribution		
Cylindric	1	0.84	Uniform	115	96.64
Apically bulbous	4	3.36	Colour rings	4	3.36
Root base			Flesh texture		
Acute	112	94.12	Crispy	63	52.94
Obtuse	2	1.68	Pithy	29	24.37
Convex	5	4.20	Fibrous	27	22.69

Table 3. Comparison of correlation coefficients for different traits of local and exotic radish germplasm.

Traits	Origin	Plant biomass (g)	Leaves (No.)	Leaf length (cm)	Leaf blade width (cm)	Root length (cm)	Root diameter (cm)
Leaves (No.)	Local	0.063					
	Exotic	0.520**					
Leaf length (cm)	Local	0.476**	-0.563**				
	Exotic	0.453**	0.247**				
Leaf diameter (cm)	Local	0.386**	-0.273**	0.596**			
	Exotic	0.703**	0.203**	0.509**			
Root length (cm)	Local	0.541**	0.126*	-0.001	-0.041**		
	Exotic	0.718**	0.562**	0.281**	0.471**		
Root diameter (cm)	Local	0.428**	0.145*	0.149*	0.196**	-0.052	
	Exotic	0.359**	-0.210**	0.049	0.397**	-0.278**	
Root weight (g)	Local	0.777**	0.257**	0.070	0.076	0.739**	0.470**
	Exotic	0.955**	0.393**	0.327**	0.658**	0.673**	0.479**

On the basis of yield and root quality, 6 genotypes were identified from the groups and data for these selected accessions are presented (Table 4). These selected accessions are suggested for further testing under wide

range of agro-ecological conditions to utilize for selection/breeding of high yielding and good quality radish cultivars.

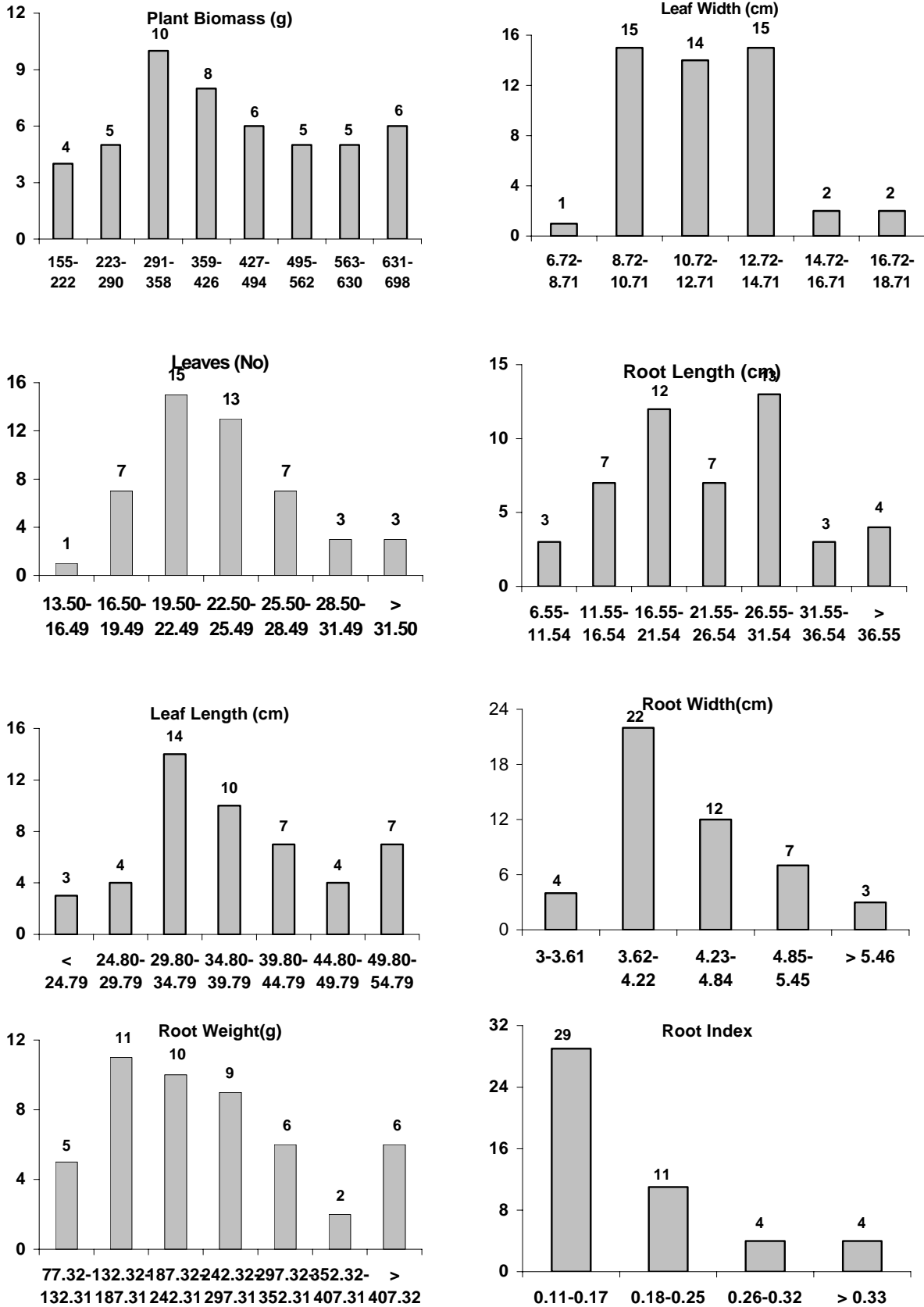


Fig. 1. Diversity in radish germplasm: Frequency distribution for different quantitative traits.

Cluster analysis: Accessions were divided into 4 clusters at 50 percent genetic distance from each other on the basis of cluster analysis (Fig. 2). The lowest mean for plant weight were found in cluster-I while cluster-III has the highest (Table 5). Since radish is grown for roots, so genotypes in cluster-III can be used to develop cultivars with more root weight. Root length, diameter and weight are economically important characters of radish. Intercluster variation is found for root length which is lowest in case of cluster-IV and highest in cluster-III. Maximum root diameter is in cluster-IV and minimum in cluster-I. Low intra cluster variation is found for both of

these characters. Significant inter and intra cluster variation is found for root yield of radish in the germplasm. Cluster-III has the maximum root weight followed by that of cluster-IV. The clustering of accessions in different groups may be useful in planning the selection and hybridization programs for crop improvement. It is also evident from the results that both Pakistani and Japanese accessions are found in all clusters. Since both local and exotic germplasm was found in all clusters, no clustering on the basis of origin was found.

Table 4. List of cultivars screened for further study.

Accession number	Country	Plant biomass (g)	Leaves (No.)	Leaf length (cm)	Leaf blade width (cm)	Root length (cm)	Root diameter (cm)	Root weight (g)
10076	PAK.	351.70	19.00	45.30	13.30	13.10	7.92	236.50
10362	PAK.	693.37	31.50	31.45	9.90	40.15	5.84	395.34
10429	PAK.	623.25	35.00	31.25	11.95	28.20	5.43	425.35
10658	JAP.	650.05	28.00	36.85	12.20	39.60	4.59	457.24
10662	JAP.	623.47	25.25	32.73	11.90	30.60	4.87	451.98
10667	JAP.	445.71	20.00	33.15	13.20	10.85	7.27	307.01

Table 5. Inter cluster variation for different quantitative characters in radish.

Cluster	No. of Acc.	Mean ± standard deviation							
		Plant biomass (g)	Leaves (No.)	Leaf length (cm)	Leaf blade width (cm)	Root length (cm)	Root diameter (cm)	Root weight (g)	Root W/L ratio
Cluster-I	15	267.01 ± 76.28	22.99 ± 4.23	29.72 ± 6.20	9.92 ± 1.00	19.74 ± 5.29	3.89 ± 0.52	164.48 ± 50.95	0.23 + 0.12
Cluster-II	23	450.72 ± 89.66	22.98 ± 3.96	43.62 ± 7.31	13.46 ± 1.84	25.10 ± 6.05	4.29 ± 0.43	235.80 ± 64.02	0.19 ± 0.06
Cluster-III	8	637.21 ± 64.00	26.80 ± 5.02	35.71 ± 4.53	11.07 ± 1.68	35.09 ± 5.88	4.96 ± 0.53	426.35 ± 53.68	0.15 ± 0.03
Cluster-IV	3	466.74 ± 126.86	19.67 ± 0.58	38.32 ± 6.28	12.93 ± 0.55	13.15 ± 2.33	7.57 ± 0.33	331.14 ± 108.73	0.60 ± 0.11

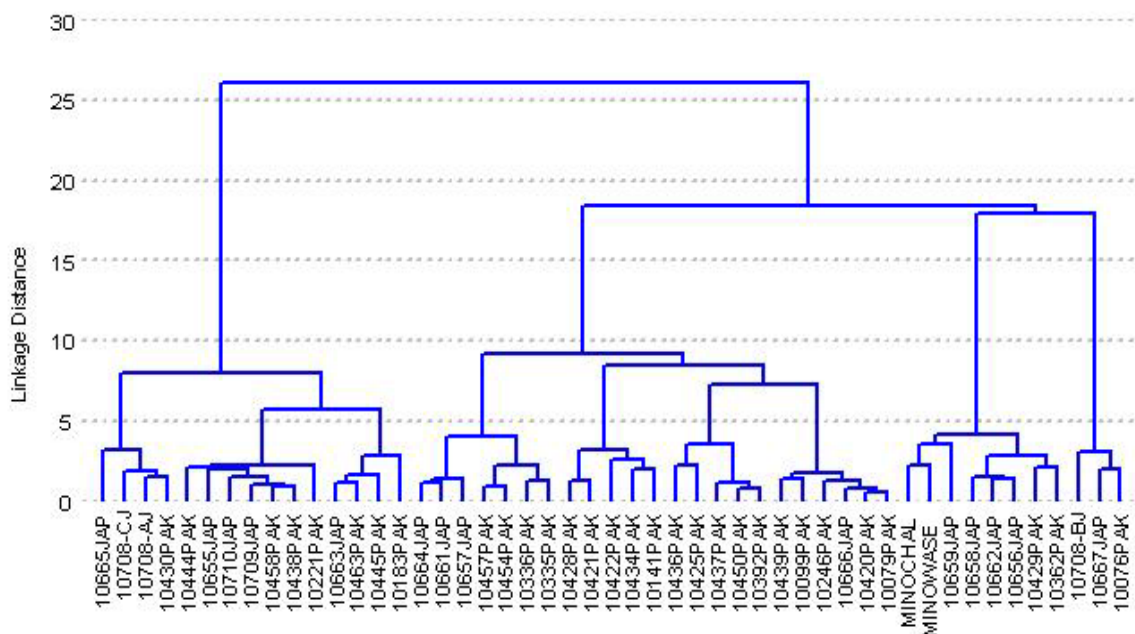


Fig. 2. Phenogram of 49 accessions of radish germplasm obtained through cluster analysis.

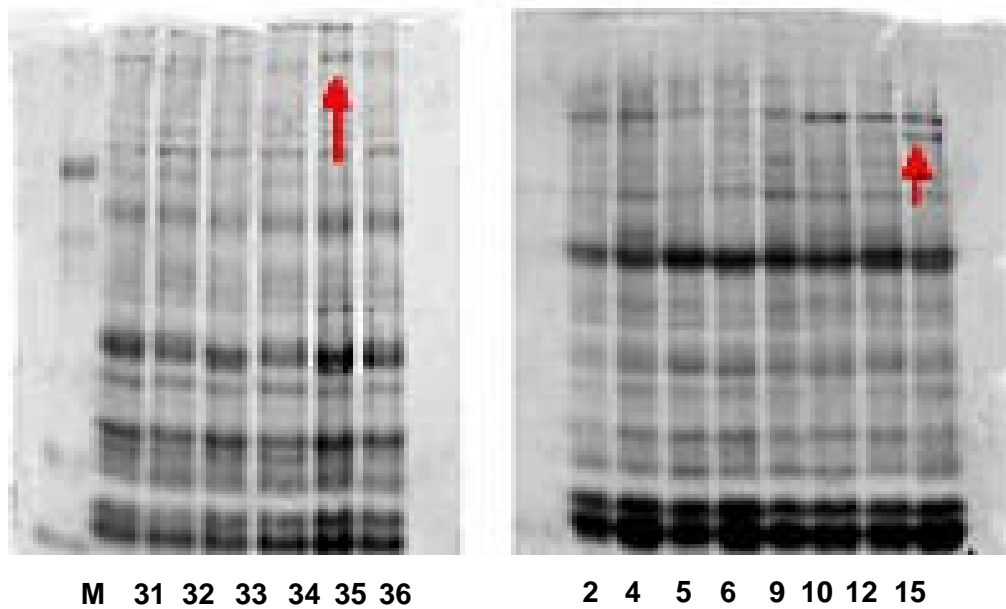


Fig. 3. SDS-PAGE protein profile polymorphic band indicated with arrow.

SDS-PAGE electrophoresis: Most of accessions showed similar pattern of protein bands through electrophoresis, however, some variation was observed in intensity of different protein bands (Fig. 3). Two unique protein bands were found at molecular weight higher than 66kd in two accessions (10428 and 10436). The low diversity for SDS-PAGE suggested the use of more biochemical markers to resolve diversity in radish. This study shows that there is low variation in radish germplasm regarding the seed storage protein. It also implies that SDS-PAGE analysis of seed storage protein is not an effective tool to study diversity.

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