



Genetic variability and divergence studies to identify and isolate superior genotypes from European radish (*Raphanus sativus*) germplasm

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ABSTRACT

The present study was carried during the winter (*rabi*) seasons of 2019–20 and 2020–21 at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh to identify and isolate superior genotypes from European radish (*Raphanus sativus* L.) germplasm. The experiment was laid out in randomized block design (RBD) comprising of 25 genotypes with three replications. These genotypes were evaluated for various horticultural and yield contributing traits, using the popular check cultivar Pusa Himani as a reference. The combined data from two years revealed significant differences among all the genotypes for the studied traits. Notably, genotypes UHF-SOL-RAD-3, UHF-SOL-RAD-1 and RKR-Sel-III exhibited higher yields compared to the check variety and performed well across most traits. The root top ratio demonstrated the highest phenotypic and genotypic coefficient of variability (34.79% and 34.64%, respectively). Traits such as total soluble solids (TSS), average root weight, root yield, gross plant weight, root top ratio (length basis), and root length exhibited moderate to high heritability along with moderate genetic advances. Based on Mahalanobis D² statistics, five clusters were identified, with a majority of genotypes falling into cluster II. Cluster III displayed the highest intra-cluster similarity (51.18%), while clusters I, III, and V demonstrated greater diversity, thereby increasing the likelihood of obtaining favourable transgressive segregants through hybridization between genotypes in these clusters.

Keywords: Genetic advance, Genotypic, Heritability, Phenotypic, Transgressive segregants

Radish (*Raphanus sativus* L.) is a diploid root vegetable with chromosome number $2n=2x=18$ belonging to the Brassicaceae family. The entire plant is edible including the leafy tops (Lamo *et al.* 2012). Radish are rich in essential nutrients such as vitamins A and C, as well as minerals like calcium, potassium, iron and phosphorus. In India radish are cultivated over an area of 207 thousand hectares, producing 3263 thousand metric tonnes (Anonymous 2021). Lu *et al.* (2008) classified cultivated radish into various varieties, such as *R. sativus* var. *niger* (Black radish), var. *sativus* (European small radish), var. *hortensis* (East Asian big long radish), var. *caudatus* (Tail-podded radish) and var. *chinensis* (Chinese oil radish). Radish cultivars can be classified into two types: Temperate or European type and Tropical or Asiatic type. Asiatic varieties are heavy yielders but have poor quality attributes and can produce both roots and seeds in tropical and temperate climates, while European radishes are small, mild flavoured, early-maturing and rich in quality attributes.

They can produce roots under both climatic conditions but seeds only in hills with chilling temperatures (Park and Kim 1985, Lakra *et al.* 2017).

In the hills of Himachal Pradesh radishes cover an area of 2.09 thousand hectares, with a production of 44.74 thousand million tonnes (Anonymous 2021). The region's temperate climatic conditions make it suitable for breeding and seed production of temperate radish. Furthermore, temperate radishes have higher nutraceutical, colourant, and antioxidant properties (Pojer *et al.* 2013, Jing *et al.* 2014). Anthocyanins present in radish have gained recognition as natural colourants as a result of their strong colouring ability, stability, distinct colour and antioxidant properties (Rahman *et al.* 2006, Matsufuji *et al.* 2007). However, a major challenge with temperate radish varieties is their low yield. Therefore, it becomes important to examine the genetic variability and divergence of different growth, quality and yield traits to identify and develop superior and high yielding varieties from diverse germplasm obtained from indigenous and exotic sources. Based on these considerations, an experiment was conducted to identify and isolate superior genotypes from European radish germplasm.

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MATERIALS AND METHODS

The present study was carried during the winter (*rabi*) seasons of 2019–20 and 2020–21 at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (altitude of 1270 m), Himachal Pradesh. The experimental material consisted of 25 diverse genotypes of European radish, along with the popular check variety Pusa Himani (Table 1). The experiment was carried out using randomized block design (RBD) having three replications. The climate at the Vegetable Farm comes under sub-temperate and sub-humid regions with cool winters. In the year 2019–20, the mean temperature ranged from 12.21–22.22°C, relative humidity from 54.00–81.80% and rainfall from 54.60–224.30 mm. Similarly, in 2020–21, the mean temperature ranged from 7.69–21.85°C, relative humidity from 57.00–77.00%, and rainfall from 5.60–171.80 mm. The soil of experimental field was sandy loam to clay loam, consisting of 46.09% sand, 32.12% silt, and 25.01% clay with a pH range of 6.85–7.04. Seeds were sown in September with a spacing of 30 cm × 10 cm in plots measuring 1.8 m × 1.0 m accommodating 60 plants/plot. Standard cultural practices recommended in the Package and Practices for Vegetable Crops developed by the university were followed. Data on various growth, quality and yield parameters were recorded each year. Data were recorded and analysed using the method given by Panse and Sukhatme (1967) for two consecutive years for different parameters, pooled and treatment means obtained were compared at a 5% significance level. The phenotypic and genotypic coefficients of variability were calculated using the formula provided by Burton and De Vane (1953). Heritability of a character is one of the most essential properties. It refers to the proportion of total variance that is attributed to the effects in average of genes and determines the degree of similarity between relatives. Further, it also expresses the accuracy of the phenotypic value for determining the breeding value. Heritability can be expressed as a fraction or may be multiplied with 100 and expressed in percentage. Heritability can be broadly classified into two groups based on their variance components and they are: 1. Broad sense heritability; 2. Narrow sense heritability. Broad sense heritability is defined as the ratio of genetic variance to that of phenotypic variance (or total variance). Narrow sense heritability is defined as the ratio of additive genetic variance to total phenotypic variance. It is more restrictive and often more useful method to estimate heritability. In present study, broad-sense of heritability (h^2b) for each characteristic was analysed as:

$$h^2b = (Vg/Vp) \times 100$$

where Vg, Genotypic variance; Vp, Phenotypic variance.

Genetic advance (GA) was determined as:

$$GA \text{ as per cent of mean} = (Vp^{1/2} \times h^2b \times k)/\text{mean}$$

where Vp, Phenotypic variance; h^2b , Broad-sense of heritability; K, Constant (2.06) at 5%

The genetic divergence in European radish germplasm was determined using Mahalanobis D^2 statistics (1936).

The Mahalanobis distance (MD) is defined as the distance between two points in a multivariate space. Generally, in a regular Euclidean space, the variables are represented on axis at right angles to each other and hence, the distance between any two points can be determined using a ruler. However, if variables are more than two and are correlated, the axis no longer remains at right angles and the measurements become difficult using a ruler. Moreover, if there are more than three variables, they can't be plotted in regular 3D space at all. Hence, Mahalanobis Distance (MD) solves this problem because it helps in measuring distances between correlated points for multiple variables. In present study, the populations were grouped into clusters using Tocher's method as mentioned by Rao (1952) and the average distances for intra-cluster and inter-cluster were calculated from the method given by Singh and Choudhary (1985). The clustering of genotypes was based on criterion that any two genotypes of same cluster and at least on an average,

Table 1 Details of genotypes used for study

Genotype	Source	Root colour
RKR-1	UHF, Nauni, Solan	White
RKR-4	UHF, Nauni, Solan	Purple
RKR-6	UHF, Nauni, Solan	Red
RKR-8	UHF, Nauni, Solan	Light purple
RKR-9	UHF, Nauni, Solan	Pink
RKR-10	UHF, Nauni, Solan	Pink
RKR-21	UHF, Nauni, Solan	Pink
RKR-26	UHF, Nauni, Solan	White
RKR-2310	UHF, Nauni, Solan	White
RKR-42	UHF, Nauni, Solan	White
RKR-42-1	UHF, Nauni, Solan	Pink
RKR Sel-II	UHF, Nauni, Solan	Pink
RKR Sel-III	UHF, Nauni, Solan	White
RKR Sel-IV	UHF, Nauni, Solan	White
UHF-SOL-RAD-1	UHF, Nauni, Solan	Pink
UHF-SOL-RAD-2	UHF, Nauni, Solan	White
UHF-SOL-RAD-3	UHF, Nauni, Solan	Pink
UHF-SOL-RAD-4	UHF, Nauni, Solan	White
UHF-SOL-RAD-5	UHF, Nauni, Solan	Purple
UHF-SOL-RAD-6	UHF, Nauni, Solan	White
UHF-SOL-RAD-7	UHF, Nauni, Solan	White
UHF-SOL-RAD-8	UHF, Nauni, Solan	Light purple
CGN-11994	Crop Genetic Resources, The Netherlands	Purple
CGN-23811	Crop Genetic Resources, The Netherlands	Black
CGN-23811-1	Crop Genetic Resources, The Netherlands	White
Pusa Himani (Check)	ICAR-IARI, Regional Station Katrain, Kullu Valley, (Himachal Pradesh)	White

shows a small deviation in D^2 value from those pertaining to two varied clusters.

RESULTS AND DISCUSSION

Variability studies: Significant differences were recorded (Table 2) among the radish genotypes for all the studied characteristics after analysing the variance, suggesting the availability of ample genetic variability that can be effectively utilized through various breeding methods. Genotype UHF-SOL-RAD-3 exhibited the highest root length (15.13 cm) and was statistically at par to UHF-SOL-RAD-2 (15.12 cm). Genotype RKR-6 recorded the highest root diameter (33.5 mm) which was comparable to UHF-SOL-RAD-5 (32.53 mm). Among crown diameter measurements UHF-SOL-RAD-1 displayed the maximum value (21.62 mm) followed by UHF-SOL-RAD-3 (21.61 mm), UHF-SOL-RAD-2 (21.58 mm) and RKR-Sel-III (21.54 mm). The genotype RKR-Sel-IV exhibited the highest root top ratio based on weight basis (4.39) while, UHF-SOL-RAD-4 recorded the maximum root top ratio based on length basis (0.47). Genotype UHF-SOL-RAD-3 showed the highest root weight (112.96 g), which was at par with the check variety Pusa Himani (109.89 g), RKR-Sel-IV (110.02 g), UHF-SOL-RAD-2 (110.75 g), RKR-Sel-III (110.89 g) and UHF-SOL-RAD-1 (111.08 g). In terms of root yield/plot and per hectare, UHF-SOL-RAD-3 attained the maximum values (6.78 kg and 301.23 q) followed by UHF-SOL-RAD-1 (6.67 kg and 296.22 q) and RKR-Sel-III (6.66 kg and 295.69 q). The maximum total soluble solids (TSS) content was obtained in genotype RKR-21 (5.05°B), whereas genotype UHF-SOL-RAD-3 exhibited

the highest ascorbic acid content (40.18 mg). Nasim *et al.* (2013), Guleria (2016), Dongarwar *et al.* (2018) and Han *et al.* (2022) also observed variability in radish germplasm for different traits, highlighting the influence of both genotype and environment on these variations. To determine the extent of phenotypic and genotypic variability, coefficients of variation was analysed (Table 2). Root top ratio (34.79% and 34.64%) exhibited high phenotypic and genotypic coefficients of variation, while average root weight (16.34% and 16.25%), root yield (16.34% and 16.25%), root top ratio (length basis) (16.63% and 16.41%) and root length (22.49% and 22.35%) displayed moderate values. Traits with higher or moderate genotypic coefficients of variation indicate greater potential for improvement through various breeding approaches. In most cases, genotypic coefficients of variability (GCV) were lower than phenotypic coefficients of variability (PCV), but variance between GCV and PCV was very less indicating a significant contribution of genetic factors in trait expression and the effectiveness of selection depending on phenotypic performance. Similar findings were reported by Jatoi *et al.* (2011), Sivathanu *et al.* (2014) and Guleria (2016), indicating considerable scope for enhancing root length, root yield, root top ratio and number of leaves through selection. The heritability data (broad sense) (Table 2) revealed high heritability values for ascorbic acid (88.98%), crown diameter (93.14%), total soluble solids (94.04%), root diameter (95.52%), root top ratio (length basis) (97.34%), average root weight (98.69%), root length (98.71%), root yield (98.85%) and root top ratio (weight basis) (99.11%). The high heritability percentages indicate the potential for improvement through selection.

Table 2 Analysis of variance, co-efficients of variability, heritability, genetic advance and genetic gain for different horticultural traits of European radish during 2019–20 and 2020–21 (pooled)

Source \ Character	df	Root length (cm)	Root diameter (mm)	Crown diameter (mm)	Root top ratio		Average root weight (g)	Root yield/plot (kg)	Root yield per hectare (q)	TSS (°B)	Ascorbic acid (mg/100 g)
					Weight basis	Length basis					
Mean sum of squares											
Replication	2	0.18	0.92	0.07	0.01	0.01	3.40	0.01	24.10	0.03	0.96
Genotype	25	19.75*	16.21*	9.50*	1.53*	0.01*	739.72*	2.66*	5260.42*	1.05*	37.99*
Error	50	0.09	0.24	0.23	0.01	0.01	2.86	0.01	20.32	0.02	1.51
Total	77	20.02	17.37	9.80	1.55	0.03	745.98	2.68	5304.84	1.10	40.46
Range		7.44	23.42	15.38	1.35	0.29	62.43		166.48	3.16	28.14
		-15.13	-33.50	-21.62	-4.39	-0.47	-112.96		-301.23	-5.05	-40.18
Mean		11.45	29.54	19.05	2.06	0.37	96.47		257.26	4.38	34.30
PCV (%)		22.49	7.98	9.56	34.79	16.63	16.34		16.34	13.8	10.78
GCV (%)		22.35	7.80	9.23	34.64	16.41	16.25		16.25	13.38	10.17
Heritability (%)		98.71	95.52	93.14	99.11	97.34	98.69		98.85	94.04	88.98
Genetic advance		5.24	4.64	3.50	1.46	0.12	32.10		85.60	1.17	6.78
Genetic gain (%)		45.73	15.69	18.34	71.03	33.35	33.27		33.27	26.73	19.76

* Significant at 5% level of significance.

TSS, Total soluble solids; PCV, Phenotypic co-efficient of variability; GCV, Genotypic co-efficient of variability.

Similar heritability patterns were observed by Singh and Singh (2012) and Guleria (2016) in radish. Nagar *et al.* (2016) also observed high heritability along with moderate genetic gains for ascorbic acid.

Genetic gain indicates the amelioration in the genotypic mean value of selected plants in comparison to the parent population. Gain in genotypic value depends on, heritability, genetic variability and selection intensity (Allard 1960). Genetic advance aids in understanding the type of gene action involved in the expression of polygenic traits. Low genetic gain values suggest non-additive gene action, while high genetic gain values indicate additive gene action. The genetic gain values in the present investigation ranged 12.25–71.03% (Table 2). The root to top ratio (weight basis) exhibited high genetic gain, while total soluble solids, average root weight, root yield, root top ratio (length basis) and root length displayed moderate genetic gain. Root diameter, crown diameter and ascorbic acid exhibited low genetic gain. However, it is important to note that higher heritability does not correspond to higher genetic advance (Johnson *et al.* 1955). High heritability along with high genetic advance suggests the influence of additive gene effects, whereas high heritability coupled with low genetic advance may indicate non-additive gene action involving dominance and epistasis (Liang and Walter 1969). Both heritability values and genetic advance as a percentage of mean (genetic gain) are useful tools for selection, providing complementary information. In this study root top ratio (weight basis) displayed high heritability coupled with high genetic advance in percent (99.11% and 71.03%) while, total soluble solids (94.04% and 26.73%), average root weight (98.69% and 31.42%), root yield (98.85% and 33.27%), root top ratio (length basis) (97.34% and 33.35%) and root length (98.71% and 45.73%) exhibited high heritability with moderate genetic advance. These findings suggest the possibility of improvement through direct selection and hybridization for these traits. On the other hand, root diameter, crown diameter and ascorbic acid demonstrated lower value for genetic advance along with higher heritability which indicates the presence of non-additive gene action which could be further exploited through, synthetics, heterosis and hybridization in European radish.

Genetic divergence studies: Genetic diversity plays a crucial role in cross-breeding programmes aimed at

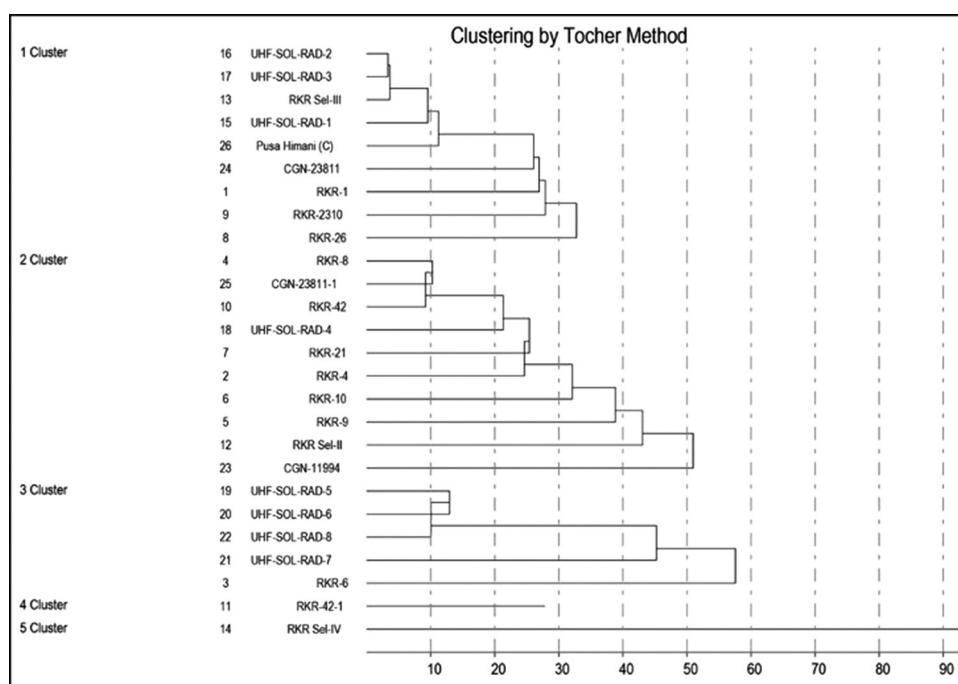


Fig. 1 Genotype clusters on the basis of genetic divergence.

improving crop species by combining genetically dissimilar parents to generate a wide range of segregants potentially leading to the development of superior crop varieties. In this particular study, 26 European radish genotypes were grouped into five clusters based on various root and quality traits (Fig. 1). The maximum genotypes (10) were grouped in cluster-II, followed by cluster-I (9), cluster-III (5) and one genotype each in cluster-IV and cluster-V (Supplementary Table 1). Interestingly, genotypes from similar geographic origin were distributed among different clusters, suggesting significant diversity in the germplasm. The intra-cluster distance is used to measure the genetic dissimilarity among genotypes within the same cluster while, the inter-cluster distance represents the genetic distance between genotypes in any two clusters. The average values of intra-cluster and inter-cluster distances (D^2) are presented in (Fig. 2). The intra-cluster distance was recorded from 0.00 (cluster-IV and cluster-V) to 51.18 (cluster-III). Whereas, the inter-cluster distance was highest (369.27) between cluster-I and V followed by III and V (335.49) and I and III (133.31). The minimum distance for inter-cluster was recorded between cluster-II and III (83.15). Clusters consisting of higher inter-cluster distances indicate that the genotypes within those clusters exhibit significant genetic differentiation. Hybridization between these distant clusters may result in a more vigorous hybrid population due to the congregation of distinct genes from the parents.

The cluster means represent the average performance of all genotypes within a specific cluster for a particular trait. Higher cluster means for a specific trait indicate higher vigour influenced by the genotypes present in that cluster. The means of cluster for all the studied traits are depicted in (Table 3). Maximum cluster means for different traits

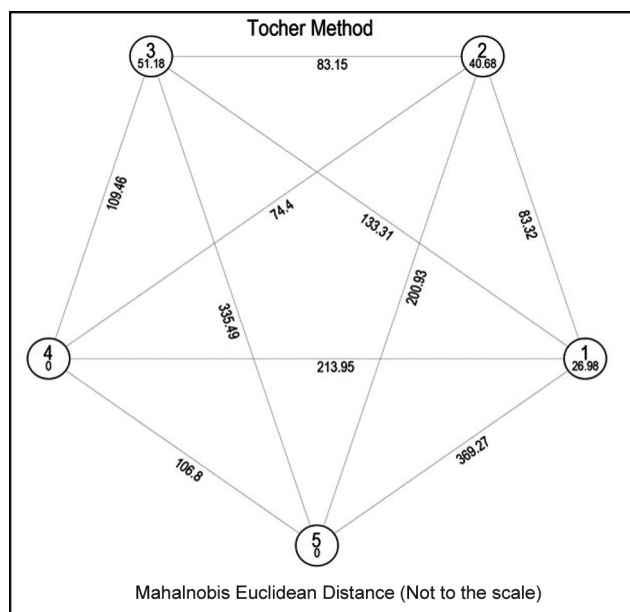


Fig. 2 Diagram showing intra and inter cluster values among different clusters.

germplasm. The larger distance among different clusters will result in wider genetic divergence between the genotypes. Whereas, crossing among genotypes of same cluster will not result in hybrids of superior quality or transgressive segregants. For future breeding programmes that include hybridization, selection of parental material should be carried out between clusters rather than within clusters. Guleria (2016), Noman (2017) and Raihan and Jahan (2019) also highlighted the essentiality of genetic divergence in radish genotypes for various traits.

Based on the 2-year study, it can be concluded that the radish germplasm exhibits significant variability which can be effectively utilized in hybridization programmes. The analysis of genetic diversity indicated that clusters I, III and V possess greater diversity thereby increasing the likelihood of obtaining desirable transgressive segregants through hybridization among genotypes from these clusters. Notably, the genotypes UHF-SOL-RAD-3, UHF-SOL-RAD-1 and RKR-Sel-III exhibited superiority as they not only outperformed the control in terms of yield but also demonstrated favourable performance across several other

Table 3 Cluster means for different characters and contribution of different traits to total divergence among 26 genotypes of radish

Trait	Cluster					Contribution (%)	Times ranked first
	I	II	III	IV	V		
Root length (cm)	13.55	11.33	8.02	8.47	14.04	9.85	28
Root diameter (mm)	28.62	29.70	30.99	26.89	31.63	5.85	19
Crown diameter (mm)	20.23	18.58	18.12	17.68	19.29	3.38	11
Root top • Weight basis	1.55	2.30	1.85	2.97	4.39	37.67	78
ratio • Length basis	0.41	0.38	0.29	0.31	0.41	1.23	4
Average root weight (gm)	107.68	99.81	68.51	88.47	110.02	15.38	32
Total soluble solids (°B)	4.40	4.30	4.32	5.02	4.57	6.46	21
Ascorbic acid (mg/100 g)	36.76	34.03	32.77	28.62	28.14	0.62	2
Root yield/ha (q)	287.15	266.16	182.68	235.92	293.38	19.56	50

were observed in cluster-V for root length (14.04) and root diameter (31.63). Cluster-I exhibited the highest crown diameter (20.23) while cluster-V displayed the highest average root weight (110.02) and yield (293.38). Cluster-V also had the highest root top ratio (weight basis) (4.39), while cluster-I and cluster-V had the highest root top ratio (length basis) (0.41). Cluster-III recorded maximum total soluble solids (5.02) and cluster-I had the maximum value for ascorbic acid content (36.76). The percentage contribution of different traits to total divergence ranged from 0.62 to 37.67% (Table 3). Root top ratio (weight basis) contributed the most (37.67%) to genetic divergence, followed by root yield (19.56%), average root weight (15.38%), root length (9.85%), total soluble solids (6.46%), root diameter (5.85%) and crown diameter (3.38%). The minimum contribution was observed for root top ratio (length basis) (1.23%) and ascorbic acid (0.62%). Therefore, traits with the highest contribution to genetic divergence can be further studied and explored to better understand the diversity present in the

horticultural traits. Furthermore, these genotypes were found to be belong to diverse clusters, highlighting the potential value of hybridization between these clusters for breeding purposes. After further multilocation testing these genotypes may be released as substitutes for existing radish varieties and can also be isolate and utilize in breeding programmes.

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