

Genetic Divergence Studies in Newly Introduced Genotypes of Lettuce (*Lactuca sativa* L.)

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Abstract: Twenty seven genotypes of lettuce including checks were planted in randomized block design at the experimental farm of the Department of Vegetable Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni - Solan during 2008. The data recorded on sixteen characters were subjected to analysis of variance. The analysis of variance revealed highly significant differences among the genotypes for all the characters studied, indicating the existence of wide genetic divergence among them. The genetic divergence in the present study observed among 27 genotypes of lettuce, showed low quantum of divergence and was grouped into six clusters. Maximum number of genotypes (8) was accommodated in cluster - III. The average inter and intra cluster divergence (D^2) values had also been calculated. The inter cluster distance was maximum between cluster II and V (), while it was minimum between cluster III and IV (). The cluster means of the various horticultural traits had been worked out. The data of cluster mean for different characters under study indicated that difference between intra cluster mean was wide for net head weight, heading percentage and yield per plot, which might be responsible for large intra cluster distances. Cluster - V showed maximum mean value for net head weight, polar diameter and yield per plot. Cluster- I had maximum value for equatorial diameter and β - carotene. Cluster - VI showed minimum value for net head weights and yield per plot. Crossing between the genotypes of maximum two clusters appeared to be most promising to combine the desirable characters.

Key words: Cluster analysis • D^2 analysis • Genetic divergence • Lettuce • *Lactuca sativa* L.

INTRODUCTION

Lettuce (*Lactuca sativa*) belongs to the Compositae (Sunflower or Daisy family). It is an annual plant native to the Mediterranean area. Lettuce is an important salad vegetable crop because of high vitamin A and minerals Ca and Fe. Variation in lettuce is mostly found in vegetative characters like leaf length, shape, colour, texture, size and heading types. Information on genetic diversity are used to identify the promising diverse genotypes, which may be used in further breeding programme. To enhance productivity, genetic restructuring of lettuce germplasm is needed to develop/identify high yielding varieties/hybrids. The usefulness of selection depends on amount of genetic variation present. Availability of large germplasm which represents diverse genetic variation is important for the progress of crop breeding and invaluable source of parental strains for hybridization and development of

improved varieties. Various methods have been employed in analysis of variation in many crop species. The value of genetic diversity by multivariate analysis [1] has been demonstrated in choosing the parental stocks for hybridization by various workers such as Malhotra and Singh [2], Bhatt [3], Katiyar and Singh [4], Dasgupta and Das [5]. This technique measures the forces of differentiation at intra - cluster and inter - cluster level and D^2 statistics groups a set of better parents on the basis of genetic divergence with the assumption that the best parents may be those showing the maximum genetic divergence. Genotypes from the same centre used to place in separate cluster indicating wide diversity among genotypes originating from the same geographic region. The existence of diversity among the genotypes was also assessed by the considerable amount of variation in cluster means for different characters. The objective of this research paper is to group a set of genetically potential parents on the basis of genetic divergence so

that a crossing programme between the genotypes of divergent groups will produce a desirable genetic variation.

MATERIAL AND METHODS

Experiment Environment and Materials: The genotypes, including check cvs. Great Lakes and Alamo - 1 (Table 1), were evaluated during the winter season in 2008-2009 at the Experimental Farm of Department of Vegetable Science, University of Horticulture and Forestry, Nauni, Solan in the state of Himachal Pradesh, India. This location, at 30°50' N latitude and 77°11'30" E longitude, is 1260 m above mean sea level and represents the mid-hill zone of Himachal Pradesh. The annual precipitation is 1000-1300 mm, with most rainfall occurring from June-September.

Seeds were sown in rows spaced 5 cm apart in raised nursery beds sized 300 × 100 × 15 cm. Developing seedlings were irrigated daily in the morning and afternoon. The experimental field was disked and leveled. About 10 Mt•ha⁻¹ of well decomposed cow manure was mixed in the soil at field preparation. The recommended fertilizer rate, kg N:P₂O₅:K₂O at 31.25:40:39 kg•ha⁻¹ was applied as calcium ammonium nitrate, single super phosphate and muriate of potash, respectively, sowing. The experiment was arranged in a randomized complete block design with three replications in 2.4 × 1.8 m plots of with a spacing 45 × 30 cm between and with in rows, respectively accommodating 32 plants/plot. In addition, 31.25 kg•ha⁻¹ nitrogen was applied in two equal installments at 30 and 60 days after sowing. Manual weeding and earthing up (pulling soil around the base of plant) was done 4-5 times until the final harvest and irrigation was applied at 15-day intervals from mid-October to mid-December and later as needed depending upon rainfall.

Ten plants/heads were randomly selected to record observations on days to marketable maturity, leaf color, number of non-wrapper leaves, gross head weight, net head weight, equatorial diameter, polar diameter, head shape index, heading percentage, yield per plot, β-carotene content, iron content, 1000 seed weight, percent seed germination, seed vigor index-I & II, total green yield and severity of bacterial soft rot. The severity of bacterial soft rot was recorded at periodical interval with the appearance of disease and severity was worked out. Non wrapper leaves were removed from head then weighed for yield and head shape index had been worked out for shape of the head [6]. Quality characters

Table 1: List of lettuce (*Lactuca sativa* L.) genotypes and their sources

Sr. No.	Genotypes	Sources
1	CGN-04508	Crop Genetic Resources, the Netherlands
2	CGN-04511	Crop Genetic Resources, the Netherlands
3	CGN-04543	Crop Genetic Resources, the Netherlands
4	CGN-04778	Crop Genetic Resources, the Netherlands
5	CGN-04925	Crop Genetic Resources, the Netherlands
6	CGN-04933	Crop Genetic Resources, the Netherlands
7	CGN-04934	Crop Genetic Resources, the Netherlands
8	CGN-04987	Crop Genetic Resources, the Netherlands
9	CGN-04988	Crop Genetic Resources, the Netherlands
10	CGN-04989	Crop Genetic Resources, the Netherlands
11	CGN-04990	Crop Genetic Resources, the Netherlands
12	CGN-05166	Crop Genetic Resources, the Netherlands
13	CGN-05167	Crop Genetic Resources, the Netherlands
14	CGN-05169	Crop Genetic Resources, the Netherlands
15	CGN-05198	Crop Genetic Resources, the Netherlands
16	CGN-09373	Crop Genetic Resources, the Netherlands
17	CGN-10944	Crop Genetic Resources, the Netherlands
18	CGN-11358	Crop Genetic Resources, the Netherlands
19	CGN-14629	Crop Genetic Resources, the Netherlands
20	CGN-14651	Crop Genetic Resources, the Netherlands
21	CGN-14688	Crop Genetic Resources, the Netherlands
22	CGN-17390	Crop Genetic Resources, the Netherlands
23	CGN-19009	Crop Genetic Resources, the Netherlands
24	CGN-19088	Crop Genetic Resources, the Netherlands
25	CGN-20721	Crop Genetic Resources, the Netherlands
26	Great Lakes	UHF, Nauni-Solan, India
27	Alamo-1	UHF, Nauni- Solan, India

like β- Carotene content and iron content had been worked out [7]. The data recorded were statistically analyzed as per design experiment described by Gomez and Gomez [8].

The genetic divergence in lettuce was estimated by Mahalanobis D² statistics. On the basis of magnitude of generalized statistical distance D (D= $\sqrt{D^2}$) values, the genotypes were grouped into different clusters as suggested by Tocber [9]. The calculation of D² values involved following steps.

- A set of uncorrelated linear combination linear (y's) was obtained by pivotal condensation of the common dispersion matrix of set of correlated variable (x's).
- Using the relationship between y's and x's the mean values of different genotypes for different characters (X₁ to X₁₇) were transformed into the mean values of a uncorrelated linear combination (y₁ to y₁₇).
- The D² values between ith and jth genotype for K characters were calculated as:

$$D^2_{ij} = \sum_{t=1}^K (y_{it} - y_{jt})$$

Group Constellation: Treating D^2 as the generalized statistical distance between a pair of populations (genotypes), all populations were grouped into number of clusters according to method described by Tocher [9]. The criteria used in clustering by this method was that, any two genotypes belonging to the same cluster, at least on an average, show a small D^2 value than those belonging to two different clusters. In other words, if genotypes V_1 and V_2 are close together and genotypes V_3 is distant from both as shown by their generalized distance then V_1 and V_2 from a cluster.

RESULTS AND DISCUSSION

Analysis of Variance: Genetic variability is the basic fundamental need for any breeding programs. Genetic improvement can be brought about by manipulating the genetic makeup of the plant for desirable characters or to remove the undesirable genes which retard, or inhibit, certain pathways. Analysis of variance (Table 2) indicated significant differences among the genotypes for all traits. These differences indicated the presence of variability and opportunity for improvement.

General Performance of Genotypes: Significant differences among the genotypes were observed for all the characters under study *viz.* days to marketable maturity, number of non - wrapper leaves, gross head weight, net head weight, heading percentage, head shape index, equatorial and polar diameter of the head, yield per plot, β -carotene content, iron content, 1000 - seeds weight, seed germination, seed vigour index-I and II and disease severity (Table 3). Among horticultural traits, comparatively wide range was observed for gross head weight (196.00 - 450.00 g), net head weight (103.33 - 250.00 g), number of non - wrapper leaves (6.46 - 14.33), heading percentage (60.54 - 89.15%), polar diameter (8.10 - 11.80 cm), equatorial diameter (5.73 - 9.10 cm), β - carotene content (1.64 - 7.06), seed vigour index-II (20.17 - 42.30) and disease severity (6.68 - 35.23). Wide range of variation for head weight in different genotypes of heading lettuce had also been reported by Thakur *et al.* [10], in cabbage for gross head weight, net head weight, heading percentage by Pearson [11], Flory and Walker [12], Swarup and Sharma [13], Jamwal *et al.* [14], Bhardwaj [15], Kumar [16], Sharma [17], Kumar [18] and Dutt [19].

CGN - 10944 gave maximum yield per plot (8.00 kg) over both the check Great Lakes and Alamo-1. This genotype also performed well for other characters like days to marketable maturity, gross head weight, net head weight, number of non - wrapper leaves and heading percentage. But found moderately susceptible to bacterial soft rot. Genotype CGN - 10944 requires further testing over years and locations for its utilization as a new variety. Besides CGN-10944, four genotypes namely CGN - 04508, CGN - 04987, CGN - 05167 and CGN - 11358 performed better for net head weight and seven genotypes namely CGN - 04543, CGN - 04987, CGN - 05167, CGN - 05169, CGN - 09373, CGN - 14629 and CGN - 19009 performed better for heading percentage over both the checks. One genotype CGN-19088 for β - carotene and iron content, CGN-05167 for 1000 - seeds weight, seed germination, seed vigour index I and II and genotypes CGN - 04778 for disease severity performed better over the checks. Besides genotypes CGN - 04778 others *viz.* CGN - 04925 and CGN - 20721 are found resistant against bacterial soft rot (*Erwinia carotovora*) as compared to checks Great Lakes and Alamo -1 which were moderately resistant.

Genotypic coefficient of variation (%) was moderate to high for gross head weight, net head weight, yield per plot, β - carotene and disease severity also has wider ranges values. Phenotypic performance would be good index for selection in lettuce for characters like gross head weight, net head weight, heading percentage yield per plots and disease severity and for quality characters *viz.* β - carotene and iron content.

Genetic Divergence Studies: In self pollinating crops like lettuce, germplasm is available in the form of a multitude of homozygous lines which can be released as genetically improved cultivars in the specific ecological regions. However, for a long term crop improvement programme, a large and diverse germplasm collection is an invaluable source of parental strains for hybridization and subsequent development of improved varieties. In this respect, various methods have been employed in the analysis of genetic variation in many crop species. The value of D^2 statistic has been demonstrated in choosing parental stocks for cross breeding. On the basis of D^2 analysis, all the genotypes (27) were grouped into six clusters (Table 4). Maximum number of genotypes (8) was accommodated in cluster - III, followed by cluster - IV (7), cluster - VI (5), cluster - II (3) and cluster I & V (2 each). A very large majority of genotypes from the Netherlands and India were grouped in different clusters. The assumption of this technique is that best genetic

Table 2: Analysis of variance for different characters in lettuce

Traits	Source of Variation		
	Genotypes (26) ^a	Replication (2)	Error (52)
Days to marketable maturity	152.86*	8.160	9.814
Number of non -wrapper leaves	13.02*	0.984	0.529
Gross head weight (g)	13.716*	3.603	1.786
Net head weight (g)	4.437*	0.535	0.387
Heading percentage (%)	135.872*	1.760	3.699
Head shape index	0.054*	0.00006	0.0008
Equatorial diameter (cm)	2.445*	0.118	0.069
Polar diameter (cm)	3.254*	0.323	0.248
Yield per plot (kg)	4.543*	0.547	0.396
β-carotene content (µg/100g)	5.357*	0.101	0.058
Iron content (mg/100g)	0.227*	0.025	0.020
1000- seeds weight (g)	0.0016*	0.0002	0.0005
Seed germination (%)	47.302*	0.405	5.215
Seed vigour index-I	0.000284*	0.000005	0.000006
Seed vigour index-II	72.955*	1.337	0.612
Disease severity (%)	67.465*	2.942	2.840

*Significant at 5% level of significance

^a values in parenthesis are degree of freedom

Table 3: Range, mean and coefficient of variation of various characters in lettuce

Characters	Mean	Standard Error	Range		Coefficient of Variation	
			Minimum	Maximum	Genotypic	Phenotypic
Days to marketable maturity	73.69	2.55	64.33	88.67	9.37	10.29
Gross head weight	339.53	34.51	196.00	450.00	18.57	22.36
Net head weight	183.55	16.06	103.33	250.00	20.02	22.71
Non wrapper leaves	9.46	0.59	6.46	14.60	21.57	22.90
Heading percentage	73.73	1.57	60.55	89.15	11.34	11.93
Head shape index	1.32	0.02	0.97	1.54	10.15	10.37
Polar diameter	9.84	0.40	8.10	11.80	10.17	11.36
Equatorial diameter	7.41	0.21	5.73	9.10	12.00	12.51
β- Carotene	3.59	0.19	1.63	7.06	36.98	37.59
Iron content	1.36	0.11	0.92	1.86	19.19	21.89
1000 seed weight	0.95	0.01	0.928	1.022	1.96	3.13
Seed germination	68.47	1.86	60.50	84.17	8.49	10.03
Seed vigour index-I	0.05	0.002	0.04	0.08	16.95	17.48
Seed vigour index-II	28.52	0.63	20.17	42.30	17.21	17.43
Disease severity	18.00	1.37	6.68	35.23	34.35	36.56
Yield per plot	5.87	0.51	3.31	8.00	20.02	22.71

Table 4: Clustering pattern of 27 genotypes of lettuce on the basis of genetic divergence

Cluster	Number of genotypes	Genotypes
I	2	CGN - 19088, Alamo - 1
II	3	CGN - 04933, CGN - 04989, CGN - 17390
III	8	CGN- 04508, CGN- 04987, CGN- 04988, CGN- 10944, CGN- 11358, CGN- 14629, CGN- 14688, CGN- 19009
IV	7	CGN- 04511, CGN- 04543, CGN- 04934, CGN- 04990, CGN- 05169, CGN- 05198, CGN- 14651
V	2	CGN- 05167, Great Lakes
VI	5	CGN- 04778, CGN- 04925, CGN- 05166, CGN- 09373, CGN- 20721

Table 5: Average intra and inter cluster distance (D^2)

Cluster	I	II	III	IV	V	VI
I	0.000	5.069	4.394	4.284	4.795	5.085
II		0.000	5.565	4.451	7.268	3.496
III			0.000	2.911	5.447	4.777
IV				0.000	6.121	3.557
V					0.000	6.735
VI						0.000

Table 6: Cluster means for characters among 27 genotypes of lettuce

Characters	Clusters					
	I	II	III	IV	V	VI
Days to marketable maturity	85.17	79.11	67.83	72.33	74.50	76.80
Gross head weight (g)	381.67	245.33	399.33	329.05	387.67	278.93
Net head weight (g)	191.67	138.89	218.33	183.57	219.00	136.47
Non wrapper leaves	11.80	13.36	8.82	8.40	9.42	8.72
Heading percentage(%)	67.96	68.70	76.83	77.06	74.64	69.11
Head shape index	1.24	1.47	1.24	1.43	1.17	1.30
Polar diameter (cm)	10.67	9.39	9.38	10.87	9.10	9.37
Equatorial diameter (cm)	8.72	6.17	7.66	7.56	8.23	6.73
β - carotene ($\mu\text{g}/100\text{g}$)	5.06	3.98	3.30	2.96	3.47	4.19
Iron content ($\text{mg}/100\text{g}$)	1.52	1.65	1.46	1.32	1.30	1.09
1000 seed weight (g)	0.95	0.96	0.95	0.95	1.02	0.96
Seed germination (%)	73.67	66.94	64.19	68.43	82.58	68.60
Seed vigour index-I	0.07	0.05	0.06	0.05	0.08	0.05
Seed vigour index-II	33.17	30.51	27.84	26.54	39.25	25.08
Disease severity (%)	16.06	14.67	22.01	20.53	15.35	11.91
Yield per plot (Kg)	6.13	4.44	7.00	5.87	7.01	4.37

parental material may be that showing the maximum genetic divergence [3]. Some genotypes from different locations are accommodated in the same cluster, indicating their close affinity. On the other hand, genotypes from same location distributed into different clusters indicate the geographical diversity may not be related to genetic diversity. The resultant six clusters showed the genetic diversity also reported by Katule *et al.* [20]. Besides helping in selection of divergent parents in hybridization, D^2 statistics also aid in the measurement of diversification and contribution of relative proportion of each component traits towards the total genetic divergence in breeding crop improvement, which has also been indicated by (Choudhary and Singh [21] and George [22].

Theoretically crossing of genotypes belonging to the same cluster will not expected to yield superior hybrids or segregants. Average inter and intra cluster divergence (D^2) values are presented in Table 5. The intra cluster distance between genotype was 0.00. The inter cluster distance was maximum (7.268) between cluster II and V, while it was minimum (2.911) between

cluster III and IV. It is expected therefore, that any cross between genotypes belonging to these diverse groups (II and V) will produce better recombinants and hybrids [23]. Such diverse genotypes characterized by maximum inter cluster distance will differ in phenotypic performance and therefore, will have genes with different magnitude of effects. In such cases, chances to obtain favourable transgressive segregants are more on the basis of results obtained. On the other hand minimum distance (2.911) was recorded between clusters III and IV, indicating minimal diversity present between these clusters. On the basis of inter and intra cluster distance, cluster V and VI may be considered as genetically more diverse and can be utilized for hybridization programme when selecting the genotypes for breeding purpose in addition to high genetic divergence. The performance of genotypes for characters with maximum contribution towards genetic divergence should also be given due consideration with respect to specific agronomic traits. Introgression of these useful genes from diverse clusters may prove useful in gene pool maintenance.

The cluster means of the various horticultural traits are presented in Table 6. Among the six clusters, the difference between inter cluster mean was wide for most of the traits in cluster V. Maximum mean gross head weight (399.33) was recorded in cluster - III, followed by cluster - V (387.67), cluster - I (381.67), cluster - VI (278.93) and cluster - II (245.33). Maximum net head weight (219.00) mean was observed in cluster - V followed by cluster - III (218.83), cluster - I (191.67), cluster - IV (183.57), cluster-II (138.89) and cluster- VI (136.47). Maximum heading percentage was observed in cluster - IV (77.06) followed by cluster - III (76.83), cluster - V (74.64), cluster - VI (69.11), cluster - II (68.70) and cluster- I (67.96). Maximum yield per plot was observed in cluster - V (7.01) followed by cluster - III (7.00), cluster -I (6.13), cluster - IV (5.87), cluster- II (4.44) and cluster- VI (4.37). However, cluster - VI exhibited minimum values for disease severity (11.91). Cluster - I recorded maximum mean value for β - carotene (5.06) followed by cluster - VI, II, V, III & IV. Iron content mean value was maximum (1.65) in cluster - IV, (1.52) in cluster - I, 1.46 in cluster-III, 1.32 in cluster-IV, 1.30 in cluster - V and 1.09 in cluster-VI. Crossing between the genotypes of maximum two clusters appeared to be most promising to combine the desirable characters. Earlier workers like Lewis Jones *et al.* [24], Fedorovo *et al.* [25], Oliveria *et al.* [26], Langton *et al.* [27], Kushwah *et al.* [28] and Lakshmiddevamma *et al.* [29] have also indicated the significance of genetic divergence.

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