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Genetic divergence and cluster analysis in garlic (*Allium sativum* L.) using D^2 statistics

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Abstract
The experiment on sixty genotypes including four check varieties (G-50, G-41, G-282 and Punjab Garlic) of garlic (*Allium sativum* L.) was conducted to work out the genetic divergence, and cluster analyses of their various attributes on yield per plant. The maximum bulb yield per plant was observed in NDG-26 (29.97 g) followed by NDG-41 (29.30 g) and NDG-5 (28.22 g) against the general mean 23.40 g. While the minimum bulb yield per plant was recorded in NDG-31 (15.13 g). In the present study, genotypes are divided into eight clusters. Cluster I had maximum number of genotypes followed by clusters II, cluster V, cluster VIII, cluster III, cluster IV, cluster VII and cluster VI. The maximum intra-cluster D^2 value observed in cluster IV. The maximum inter-cluster distance was observed between cluster VII and cluster VIII. The cluster I showed highest mean for number of cloves per bulb and cluster IV showed highest mean for weight of clove. A per cent contribution showed that plant height was found for highest contribution followed by length of leaf, number of cloves per bulb and total soluble solids for total divergence among the available genotypes of garlic.

Keywords: Garlic (*Allium sativum* L.), genetic diversity (D^2), cluster and bulb yield

Introduction
Garlic (*Allium sativum* L.) is classified under the class monocotyledone 2n=2x=16, and belongs to the section Porrum of the family Alliaceae. It is known as *Lahsun* in Hindi, is the second important bulb crop after onion grown in India. It is also important foreign exchange earner and used as spices & condiments. Garlic is rich in protein, phosphorus, potassium, calcium and carbohydrates and also considered as "Nectar of Life" in Ayurveda. A compound bulb contains the edible product of this crop. Garlic is used all over the world for flavouring, different kinds of food materials and as condiments, notable in chutneys, pickles, curry powders, curried vegetables, meat preparation, tomato ketchup in medieval Europe, it is widely used for distinguishing the smell and flavour of salted meat, fish in the Philippines, much of Eastern Asia and other parts of tropics. The dehydrated garlic in powdered or granulated form is replacing the fresh bulbs for industrial and home use in many countries.

Central Asia is the primary centre of origin of garlic followed by Mediterranean region (Thompson and Kelly, 1957). Garlic is cultivated from long ancient times. Its wild ancestor *Allium longicuspis* Regel is native to Central Asia. It is well known in pre-dynastic cemeteries in Egypt before 3,000 B.C. It was reached to China and India and grown widely since a long times and carried out to Western hemisphere by Spanish, Portuguese and French and widely grown & used in Mediterranean climates. The major garlic growing countries are France, Spain, U.S.A. Brazil and Egypt. Asia contributes major parts in total world production. It was used in England as early as first half of the 16th Century. It is frost resistant vegetable crop grown throughout the India. China is the leading country in area & production of garlic followed by India.

In India, the total area covered under garlic is about 0.26 Million hectare with production of 1.42 Million tonnes and their productivity is 5.43 tonnes per hectare of bulb. (Anonymous, 2015) [1]. Madhya Pradesh is the leading state in garlic production, its share, 0.06 Million hectare area with 0.27 Million tonnes production. The important garlic growing states are Gujarat, Maharashtra, Uttar Pradesh, Andhra Pradesh, Orissa, Tamil Nadu and Rajasthan.

Garlic has higher nutritive value than other bulb crops. A colourless, odourless, water-soluble amino acid known as *Alliin* is present in uninjured garlic cloves. On injury of the cells, an enzyme, allinase comes in contact with Alliin and causes its breakdown into a sulphur containing product allicin (Diallylthiosulfinate). Allicin is the antibacterial substance of garlic and has the typical odour of fresh garlic.

It is unstable and breaks down into the strong smelling constituents of garlic oil. The alliin of *Allium sativum* contains an allyl radical from which the pungent Diallyl di-sulphide, is derived, which is responsible for pungency in garlic. Garlic contains 0.1-0.4% essential oil. The chief constituents of the oil are diallyldi-sulphide (60%), diallyltrisulphide (20%), allyl-propyl disulphide (6%) and small quantity of diethyl disulphide.

Garlic has higher nutritive value than other Allium bulb crop. Garlic is the rich source of carbohydrate 29.0 g, protein 6.3 g and phosphorus 310 mg of 100g fresh weight. It is rich source of thiamine 0.16 mg and riboflavin 0.23 mg of 100 g fresh weight. It contains fair amount of calcium 30.0 mg of fresh weight. Ascorbic acid content is quite higher in green garlic.

Garlic has good medicinal properties. It is carminative, gastric stimulants, helps in the digestion of food & absorption of nutrient from it. The inhalation of garlic oil or garlic juice has generally been recommended in case of pulmonary tuberculosis, rheumatism, sterility, impotency, cough and red eyes. Augusti (1977) [2], reported that allicin, which has a hypo-cholesterolemic action, is present in aqueous extract of garlic and reduces the cholesterol concentration in human blood which is responsible for heart attack. Garlic juice is also used as medicine in various skin diseases.

The garlic extracts has nematicidal, bactericidal and fungicidal properties. The antioxidant potential of garlic is one of the great interest in connection with the anti-atherosclerotic & cardio protective effects observed epidemiologically and clinically. Garlic possesses insecticidal action. Repellent property of garlic has been demonstrated by Bhuyan *et al.* (1974) [3]. A formulation containing 1% garlic extract gave protection to persons against mosquitoes & black fly. According to Sukul (1974) [12], extract of garlic showed strong nematicidal action of killing *Meloidogyne incognita* and other species of soil nematodes. Sharma (1977) [9], reported, that a crude extract of garlic clove have antibacterial activity against gram positive & gram negative bacteria.

Most of the available cultivars of garlic are white coloured, but sometimes pink or red types are also occurred. Kenmochi and Katayama (1975) [4] reported, that anthocynin pigment in garlic & onion were very similar and the major pigment in each was *cyaniding*-3-*glucoside*.

Garlic plants have flat, grayish-green leaves, which grow 1-2 feet. It is sexually sterile plants, the plants send up slender stalks, which produce round white bulb. Single bulb is composed of many sections called as cloves, which are held together by parchment like covering. It is annual for bulb and biennial for seed production. Histogenesis of seed stalk of inflorescence was studied by Kothari and Shah (1974) [6]. It was observed that the seed stalk bears a terminal inflorescence which in turn bears bulbils instead of flowers. The shoot becomes flat and finally aborts after the development of bulbils in the inflorescence.

Garlic is vegetatively propagated by cloves, and those cultivars that still bolt, by inflorescence bulbils. Some modern cultivars produces flowers mixed with bulbils, but the flowers never set seeds. Garlic thus presents an interesting problems as to the origin of many cultivars, differing in maturity, bulb size, clove size and number, scale colour, bolting and presence and absence of flowers, it is not known how much variation due to bud mutation has arises after garlic became vegetatively propagated.

Yield is very complex characteristics controlled by several yield contributing components and it is highly influenced by environmental factors, consequently estimates of heritability and genetic advance are useful for selection. Estimation of correlation co-efficient among the yield contributing characters is necessary to understand the direction of selection and maximize yield. Path coefficient provides an effective means of entangling direct and indirect causes of association of selection and measures the relative importance of each causal factor.

Beside these above parameters Mahalanobis D^2 analysis was also utilized which helps in the assessment of genetic diversity among the genotypes and the contribution of each character to the total diversity. Greater the genetic distance, there are better chances for obtaining desirable hybrids or segregates after hybridization between diverse groups.

Materials and Methods

The study was designed to work out the genetic divergence, and cluster analyses of their various attributes on yield per plant among 60 garlic genotypes including four check varieties (G-50, G-41, G-282 and Punjab Garlic) at field experiment under present investigation was conducted during *Rabi* 2014-15 at the Main Experiment Station, Vegetable Science, N. D. University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad (U.P.) India. The experiment was laid out in Augmented Block Design, with plot size of 2.0 × 0.60 m^2 with distance of 30 cm row to row and 10 cm plant to plant. Observation were recorded on plant height (cm), number of leaves per plant, length of leaf (cm), width of leaf (cm), neck thickness of bulb (cm), diameter of bulb (cm), length of bulb (cm), bulb yield per plant (g), number of cloves per bulb, length of clove (cm), weight of clove (g), diameter of clove (cm) and total soluble solids (%).The study of genetic divergence of sixty genotypes was done through Mahalanobis D^2statistics as described by Rao (1952).

Results and Discussions

Distributions of genotypes in different clusters

The studies on genetic divergence among sixty genotypes of garlic were carried out by using Mahalanobis D^2 statistics'. In present investigation sixty genotypes of garlic were grouped in eight distinct non- over lapping clusters. This indicates presence of considerable diversity in the genotypes. The major clusters in the above mentioned genetic divergence analysis contained frequently the genotypes of heterogeneous origin. Although the genotypes of same origin or geographic region were also found to be grouped together in the same cluster. The instances of grouping of genotypes of different origin or geographic region in same cluster were frequently observed. This suggested that there is no parallelism between genetic and geographic diversity.

The highest number of genotypes appeared in cluster I which contained fifteen genotypes, followed by cluster II having fourteen genotypes, cluster V having eleven genotypes and cluster VIII having seven genotypes, cluster III having six genotypes followed by cluster IV having four genotypes and cluster VII having two genotypes followed by cluster VI, which have one genotype respectively among all the clusters table-1. In the present study, genotypes are divided into eight clusters. Cluster I had maximum number of genotypes followed by clusters II, cluster V, cluster VIII, cluster III, cluster IV, cluster VII and cluster VI. Similar results have

been reported by Khar *et al*. (2006) [5].

Average intra and inter- cluster distances

The intra and inter - cluster distance among different clusters are given in table-2. The intra-cluster D^2 values ranged from 0.00 (cluster VI) to 26.30 (Cluster IV). The maximum inter-cluster value (60.30) was found between VII and VIII. The minimum inter- cluster value was found between I and II (17.92) indicates that this group is more diverse.

The maximum intra-cluster D^2 value observed in cluster IV. The maximum inter-cluster distance was observed between cluster VII and cluster VIII, which suggested that members of these two clusters are genetically very diverse to each other. Fallowed by cluster VI to cluster VII, cluster IV to VII, cluster IV to VI, cluster II to VI and cluster II to VII were very high inter-cluster value. The minimum inter-cluster D^2 value was recorded in case of cluster I and cluster II followed by cluster II and cluster III. The higher inter-cluster distance indicated greater genetic divergence between the genotypes of these clusters, while, lower inter cluster values between the clusters suggested that the genotypes of the clusters were not much genetically diverse from each other. Similar results were also reported by Khar *et al*. (2006) [5].

Cluster means

The mean performance for all the characters in different clusters is presented in table-3. Cluster VI showed highest mean for plant height (66.999), number of leaves per plant (10.377). Cluster V showed highest mean for length of leaf (41.591). Cluster VII showed highest mean for width of leaf (3.345), length of bulb (5.655), bulb yield per plant (27.090), length of clove (3.698) and total soluble solids (39.849).

Table 1: Clustering pattern of sixty garlic genotypes on the basis of Non-hierarchical Euclidean Cluster Analysis for 13 characters

Cluster Number	Number of genotypes	Genotypes
I	15	NDG-1, NDG-56, NDG-20, NDG-21, NDG-22, NDG-2, NDG-38, NDG-55, NDG-3, NDG-25, NDG-7, NDG-16, NDG-17, NDG-19, NDG-49
II	14	NDG-5, NDG-8, NDG-18, NDG-9, NDG-48, NDG-6, NDG-14, NDG-40, NDG-12, NDG-28, NDG-15, NDG-35, NDG-32, Punjab Garlic
III	6	NDG-4, NDG-11, NDG-23, G-282, NDG-24, G-41
IV	4	NDG-26, NDG-39, NDG-33, NDG-41
V	11	NDG-10, G-50, NDG-46, NDG-27, NDG-42, NDG-51, NDG-45, NDG-50, NDG-44, NDG-47, NDG-43
VI	1	NDG-54
VII	2	NDG-52^{x}, NDG-53^{x}
VIII	7	NDG-13, NDG-29, NDG-34, NDG-36, NDG-37, NDG-30, NDG-31

Table 2: Estimates of average intra and inter-cluster distances for 8 clusters in garlic

	I Cluster	II Cluster	III Cluster	IV Cluster	V Cluster	VI Cluster	VII Cluster	VIII cluster
I Cluster	16.21	17.92	23.14	33.17	20.81	44.82	45.20	31.53
II Cluster		12.40	19.78	30.55	22.31	50.07	48.71	24.24
III Cluster			14.50	29.78	28.90	43.24	47.08	26.83
IV Cluster				26.30	35.05	53.07	53.98	43.13
V Cluster					17.09	40.19	47.87	36.45
VI Cluster						0.00	59.34	45.12
VII Cluster							8.86	60.30
VIII Cluster								21.54

Table 3: Cluster means for 13 characters in Garlic

Characters	Plant height (cm)	Number of leaves per plant	Length of leaf (cm)	Width of leaf (cm)	Diameter of Bulb (cm)	Length of Bulb (cm)	Neck Thickness of Bulb (cm)	Bulb Yield per plant (g)	Number of cloves per Bulb	Length of Clove (cm)	Weight of Clove (g)	Diameter of Clove (cm)	T.S.S. (%)
I Cluster	62.32	8.07	35.21	1.26	3.65	5.40	1.48	24.26	22.52	2.44	1.06	1.26	38.12
II Cluster	58.99	8.03	35.22	1.22	3.46	4.95	1.70	23.95	21.59	2.53	1.11	1.78	37.96
III Cluster	56.21	7.77	33.09	1.37	4.17	5.12	1.45	22.08	17.19	3.35	1.31	1.61	37.22
IV Cluster	62.41	7.68	37.65	1.43	3.61	5.28	1.62	26.88	19.05	2.81	1.91	1.49	37.17
V Cluster	65.58	8.99	41.59	1.18	3.74	5.38	1.51	23.93	22.42	2.64	1.10	1.44	35.00
VI Cluster	66.99	10.37	36.91	1.02	2.84	5.14	1.03	15.38	10.46	3.63	1.44	0.86	34.80
VII Cluster	54.09	10.27	38.47	3.34	3.31	5.65	1.32	27.09	21.56	3.69	1.23	1.12	39.84
VIII Cluster	56.35	7.56	33.86	1.59	3.26	4.36	1.64	18.90	16.31	2.45	1.13	1.52	33.75

Cluster III showed highest mean for diameter of bulb (4.176). Cluster II showed highest mean for neck thickness of bulb (1.709) and diameter of clove (1.786).

Cluster I showed highest mean for number of cloves per bulb (22.522). Cluster IV showed highest mean for weight of clove (1.911).

Cluster six showed maximum mean values for the plant height and number of leaves per plant. Cluster V showed highest mean for length of leaf. Cluster VII showed highest mean for width of leaf, length of bulb, bulb yield per plant, length of clove and total soluble solids (TSS). Cluster III showed highest mean for diameter of bulb.

Cluster II showed highest mean for neck thickness of bulb and diameter of clove, whereas cluster I showed highest mean for number of cloves per bulb and cluster IV showed highest mean for weight of clove. Similar finding were also reported by Singh *et al.* (2013) [11].

Per cent contribution of thirteen characters towards total genetic divergence in garlic

A perusal of per cent contribution is presented in table-4. showed that width of leaf, diameter of bulb, length of bulb, neck thickness of bulb, length of clove, weight of clove and diameter of clove and number of leaves per plant (0.11%), contributed very low towards the divergence while, plant height was found for highest contribution (35.42%) followed by length of leaf (23.39%), number of cloves per bulb (22.49%), total soluble solids (10.73%) and bulb yield per plant (7.85%) for total divergence among the available genotypes of garlic.

A perusal of per cent contribution table-4. Showed that plant height was found for highest contribution followed by length of leaf, number of cloves per bulb and total soluble solids for total divergence among the available genotypes of garlic, while number of leaves per plant, bulb yield per plant, diameter of bulb, contributed very low towards the divergence. Similar results were reported by Patil *et al.* (2013) [8] and Shashidhar and Dharmatti (2005) [10].

Table 4: Percent contribution of thirteen characters towards total genetic divergence in garlic

S. No.	Source	Contribution (%)
1	Plant height (cm)	35.42
2	Number of leaves per plant	0.11
3	Length of leaf (cm)	23.39
4	Width of leaf (cm)	0.00
5	Diameter of Bulb (cm)	0.00
6	Length of Bulb (cm)	0.00
7	Neck Thickness of Bulb (cm)	0.00
8	Bulb Yield per plant (g)	7.85
9	Number of cloves per Bulb	22.49
10	Length of Clove (cm)	0.00
11	Weight of Clove (g)	0.00
12	Diameter of Clove (cm)	0.00
13	T.S.S. (%)	10.73

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