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Study of morphological and molecular characterization of garlic (*Allium sativum* L.)

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ABSTRACT : Genotypic and phenotypic co-efficients of variation were high for average weight of clove, yield per plant, cloves per bulb and plant height. Width of clove, plant height and length of leaves, yield per plant, cloves per bulb, average weight of clove exhibited high heritability coupled with high genetic advance as per cent of mean. These characters are governed by additive gene effects. It was also concluded that selection on the basis of these characters will be more useful for the improvement of this crop towards attaining higher yield. Correlation co-efficient studies showed that phenotypic correlation co-efficients are higher than genotypic correlation which indicates the inherent association among the characters dependent of environment influence. Yield per plant had a positive and highly significant correlation with plant height, length of leaves, average weight of clove, equatorial diameter, polar diameter and clove/bulb which implies that these characters were contributing to bulb weight and bulb yield/plant. All the combination of traits should be considered while selecting for high yielding genotypes. Improvement of bulb weight per plant may be achieved by exercising direct selection of plant height, length of leaves, weight of cloves, equatorial diameter of bulb, clove weight, number of cloves per bulb, polar diameter of bulb and clove length as these exhibited significant positive direct effect on bulb weight per plant coupled with high heritability and high genetic advance as per cent of mean. Characters had correlation co-efficient value at par with their direct effect on bulb yield per hectare. In Mahalanobis D² analysis, On the basis of Mahalanobis D² values, all the 27 genotypes were grouped under study were grouped into six clusters. Cluster III (8), cluster V (6) and cluster VI (6) contained maximum number of genotypes and cluster I (4) and cluster II (2) comprising minimum number of genotypes and cluster IV contained single genotype.

KEY WORDS : PCR, SSR, Cluster, EDB, Genotype, Phenotype

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Garlic is a monocotyledonous vegetable that has its origin in Central Asia (Kazakhstan), with secondary centers of diversification in China and the Mediterranean area. Garlic is the second most important bulb vegetable grown and used as spice and flavouring agent for many foods. "*Allium* is the largest and the most important representative genus of the

Alliaceae family that comprises 700 species, widely distributed in the Northern hemisphere, North America, North Africa, Europe and Asia (Tsiaganis *et al.*, 2006). Garlic is a diploid species of obligated apomixes, therefore, its reproduction is vegetative. Although some garlic plants found in the Campania region of Italy were shown to be tetraploid, although some cultivars might be

triploid. Garlic has a very large genome 33.5pg/2C. Garlic is grown in India in an area of 262.06ha having a production of 1425.46MT and productivity of 5.4MT/ha. Whereas in Bihar garlic is grown in an area of 4.25ha area having a production 4MT and productivity 0.94MT/ha (NHRDF 2014-15). Madhya Pradesh is the leading state accounting for more than 27 per cent of area and 25 per cent of the total production of garlic with average yield of 4.5MT/ha. West Bengal has the highest productivity at 11.94T/ha. The name *Allium sativum* is derived from the Celtic word “all?” meaning burning or stinging and the Latin “sativum” meaning planted or cultivated. The English word, garlic is derived from the Anglo-Saxon gar- leac or spear plant, referring to its flowering stalk. Garlic produces bulbs and has a floral-scape stem. The inflorescences seldom set seed but often develop bulbils on the top of the inflorescences. Garlic bulbs are divided into cloves that together with the bulbils are the propagules of the crop. It is also being used as green stalks and young leaves are eaten fresh or cooked and furthermore, large quantities of garlic used for pharmaceutical purposes. In botanical terms the edible part of garlic is composed of garlic cloves. Garlic contains about 30-35 per cent dry matter, 6-7 per cent protein, 0.2 per cent lipids, 21-28 per cent carbohydrates, 0.7-0.9 per cent fibre, 1.1-1.4 per cent ash matter and vitamins, especially B₁, B₂, B₆ and C. Garlic also contains antibiotics like garlicin and allistatin and number of enzymes, amino acids, universal substances, including trace elements. Garlic is consumed directly, or to add flavour to food, in smoked-meat products and for the production of medicaments. At present it is valued for its content of essential oils. The concentration of nitrates in vegetables is an important qualitative criterion: sulphur being a component of the enzyme nitrite reductase, which is responsible for the reduction of NO₂ in chloroplasts. The *Allium* genus of such vegetables is characterized by a composition that is high in flavonols and organo sulfur compounds. Furthermore, animal and in vitro studies provide evidence of the anti carcinogenic potential of several bioactive compounds in *Allium* vegetables. Garlic used worldwide as a food and folk medicine since ancient times, is one such natural food. Likewise, garlic has been used for thousands of years as an herbal remedy. Modern studies have confirmed a wide variety of benefits from garlic, including reduction of serum cholesterol, inhibition of platelets aggregation, antiviral activity, stimulation of immune responses and even

antitumor activity, also garlic has been reported to be an effective form of treatment for a variety of malignancies over the years. Garlic is known to be of therapeutic value in the treatment of infections caused by bacteria, fungi, virus and protozoa, natural antibiotic, anticarcinogenic activity, atherosclerosis by lowering the blood cholesterol. Active ingredients are allicin and diallyl disulfides. In fact, *Allium* species are a rich source of phytonutrients, useful for the treatment or prevention of a number of diseases, including cancer, coronary disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract like colic pain, flatulent colic and dyspepsia. Other compounds which contain sulphur are e.g. tripeptide deethylglutathione (antioxidant and precursor of phytochelatin which are also able to detoxify some heavy metals), ferredoxin, sulpholipids, glucosinolates, nitroreductase, vitamins B₁ and others. Garlic is rich in sugar, protein, fat, calcium, potassium, phosphorus, sulfur, iodine and silicon, in addition to vitamins. Its pungent flavour makes it used mainly as a spice, seasoning and flavouring for foodstuff involving both green tops and bulbs. For centuries, garlic has been clonally propagated, which may be speculated to result in a bottleneck for genetic variation in garlic. However, recent studies genetic studies revealed that garlic displays a wide range of variation under various ecological conditions and some germplasm have adapted to specific environments through artificial and natural selection. Garlic germplasm have normally show wide variations in characteristics such as bulb weight, coat layer, leaf length, growth habit and stress resistance. So, evaluation of garlic genetic resources both by morpho-agronomic traits or molecular markers will make us to understand the variation between accessions and select out those with our interested character for breeding programme. The information on the native and magnitude of genetic variability present in the genetic stocks, heritability and genetic advance among various traits are of considerable use in future breeding programmes. The direct and indirect effects of these different components on yield are measured by path coefficient analysis. The characters of economic importance are generally quantitative in nature and exhibit considerable degree of interaction with the environment. Thus, it becomes necessary to compute variability present in the material and its partitioning into genotypic, phenotypic, and environmental effects. Characterization of germplasm materials helps to ensure

an efficient and effective use of genotypes for further crop improvement. Mahalanobis D^2 analysis helps in assessing the diversity among the genotypes and to select the divergent parents for future breeding programmes. Currently, such assessment is mainly based on a small number of phenotypic traits. However, environmental conditions may affect their expression and so assessing only morphological traits may not reflect the genetic diversity available. Marker aided selection is a very useful technique. It is the only option to reduce the time, space and cost of varietal development. Introduction of DNA-based molecular markers have made an important contribution to develop genetically engineered crops. They are widely used for the process of genome analysis. The discovery of PCR (Polymerase chain reaction) was a milestone in this area that facilitated the marker-assisted selection of desirable genotypes. It opened new dimensions to various efforts of breeding and marker-aided selection that could reduce the time span of developing new and better varieties. Keeping the above facts in view, the experiment was conducted to determine genetic diversity of twenty seven garlic accessions using morpho-agronomic traits and molecular markers. This result of this study will provide reproducible data across laboratories and identification of potential duplicates, reducing cost in germplasm maintenance and promoting faster breeders' selection and exchange of materials. In the present study of Morphological and Molecular characterization of garlic (*Allium sativum* L.)", was carried out with the following objectives. (i) To characterize diverse genotypes on morphological and molecular basis (ii) To study the variability in the existing genotypes (iii) To establish inter relationship among various attributes under study.

RESEARCH METHODS

The details of experimental materials and method employed in the present investigation entitled

“Morphological and Molecular characterization of garlic (*Allium sativum* L.)” is as follows:

Experimental site and location :

In the present investigation was carried out at maize research farm of Bhagwant University Farm Ajmer Rajasthan, India during *Rabi* season 2015-16 and molecular work was performed at Molecular Biology Laboratory (MBL), Department of Genetics and Plant Breeding, College of Agriculture, Bhagwant University Ajmer, Rajasthan, India, during *Rabi* season 2015-2016. Which is geographically situated between 26°C' N latitude to 76.63°C Elongitude at 46m above mean sea level. All the necessary facilities for cultivation of successful crop including field preparation, inputs, irrigation facilities and labours were provided by department of horticulture, Bhagwant University, Ajmer.

Experimental materials :

The present study consisted of 27 garlic genotype collected from different districts/regions of Bihar. List of genotypes which are shown in Table A.

Layout plan of work :

The investigation was statistically laid out in the Randomized Block Design (RBD) with twenty seven genotypes, replicated thrice (illustrated in Table B).

Observations for morphological characters:

Plant height (cm) :

The plant height was measured from the neck of the bulb to the tip of the longest leaf of four randomly selected plants by using measuring scale at 30 days before harvest and mean values was expressed in cm.

Leaf length (cm):

The total length of three longest leaves from base

Table A : List of genotypes						
	Garlic genotype		Source	Garlic genotype		Source
4	RAU-G5	543	Local collection from	BRG-7	Local collection maintained	
BRG-14	IC-375119	WG-2	DOGR maintained at BU,	BRG-13	at BU, Ajmer	
G-232	G1	650	Ajmer	BRG-9		
IC-345585	IC-290440	WG-7		BRG-10		
453	527	RG-464		BRG-3		
644	M-175	WG-73		BRG-8		
569	WG-7-1	IC-375107				

Table B : Randomized Block Design (3 replication) for 27 genotypes of garlic					
	R ¹		R ²		R ³
4	BRG-14	BRG-7	453	G-232	569
G-232	BRG-7	644	569	BRG-13	BRG-9
BRG-13	IC-345585	BRG-14	WG-73	4	644
453	BRG-9	RG-464	4	IC-375107	BRG-7
644	569	WG-2	650	IC-345585	BRG-8
WG-73	WG-7	G-232	IC-375107	BRG-14	M-175
RG-464	IC-375107	BRG-13	WG-7-1	BRG-10	WG-73
543	WG-2	BRG-9	BRG-3	RAU-G5	WG-7
650	BRG-10	BRG-8	IC-290440	IC-375119	RG-464
RAU-G5	IC-375119	543	M-175	527	BRG-3
G1	BRG-3	WG-7	527	WG-2	453
IC-290440	527	G1	BRG-10	650	IC-290440
BRG-8	M-175	IC-345585	IC-375119	WG-7-1	G1
WG-7-1		RAU-G5			543

Table C: Randomized Block Design carried out for 27 genotypes of garlic		
Design- RBD	Planting spacing-10 cm × 15 cm	Plot size-2m x 1.5m
No. of replications-03	Check varieties- G1 and G282	Number of plant per plot-200
Sowing season- Main season (<i>Rabi</i> season)		

to top was measured on each randomly selected plant by using scale and mean values was expressed in cm.

Leaf number/plant :

Number of leaves was counted of three randomly selected plants and mean value was expressed.

Breathe of leaf (cm) :

Breath of leaves was measured with the help of measuring scale at 30 days before harvest of crop and mean value was expressed (cm).

Neck thickness (cm):

Thickness of neck of bulb was measured with the help of verniar-caliper and mean value was expressed (cm).

Polar diameter (cm):

The bulb length was measured from base to neck constriction with the help of verniar-caliper and he value expressed in (cm).

Equatorial diameter of bulb (cm):

The bulb maximum circumference at the broadest

point was measured with the help of verniar-caliper and the value expressed in (cm)

Bulb weight per plant (g):

Crop was harvested; bulbs were cleaned. Cleaned bulbs were weighed in an electronic balance and the mean values were expressed in grams.

Number of cloves per bulb:

The cloves were counted on the basis of randomly selected three bulb, selected cloves are numbering with the help of manually per bulb and counted average.

Length of the clove (cm):

The total length of three cloves from base to tip was measured on each randomly selected by verniar-caliper and averages was calculated.

Clove weight (g):

The cloves weight were counted on the basis of randomly selected three cloves, selected cloves are weighing with the help of weighing meter and counted average weight of cloves in gram.

Width of clove:

The total width of three cloves from broadest point was measured on each randomly selected by vernier-caliper and averages was calculated.

Plant growth habit :

Habit of garlic plants is erect/spreading.

Colour of leaves :

Colour of leaves of garlic based on garlic colour chart, green, light green and dark green.

Bulbils :

Presence and absence.

Bulb texture :

Thick or thin.

Bulb skin colour :

White/creamy white/pinkish.

Clove skin colour :

White/ white pinkish.

Clove flesh colour :

Whitish or yellowish.

Pungency :

Pungency of garlic based on hedonic point nine scale

Molecular marker :**DNA extraction :**

Garlic genomic DNA was isolated from 20-30 days old plants leaf of 27 genotypes using the standard cetyltrimethyl ammonium bromide (CTAB) method

(Doyle *et al.*, 2009) For this purpose 115mg leaf tissue was grinded in mortar and pestle with 1ml of 2x DNA extraction buffer [100mM Tris-HCl, 1.4M NaCl, 30mM EDTA, and 2% (w/v) CTAB pH; 8.0]. A pinch of polyvinylpyrrolidone (PVP) and 30 μ l of β -mercaptoethanol were added. Again 1 ml of 2x DNA extraction buffer was added until sample was finely grinded. Then sample was taken in two micro-centrifuge tubes. Samples were kept for inoculation in water bath at 65°C for 45min with occasional shaking at 10min interval. After incubation, 750 μ l of chloroform: isoamyl alcohol (24:1, v/v) was added and shaken well for 10min and centrifuged at 10,000rpm for 5min. Upper organic phase was carefully separated and transferred to fresh tube. This was subjected to again extraction in presence of chloroform: isoamyl alcohol. Upper organic phase was collected and equal volume of isopropanol was added. After mixing, the sample was centrifuged for 10min at 10,000rpm. Supernatant was discarded and 500 μ l of 70 per cent of chilled ethanol was added to the pellet for washing the pellet property. Following centrifugation for 5min at 10,000rpm, the supernatant was discarded and sample was air dried. Finally the pellet was dissolved in 30 μ l of 10:1 TE (50mM tris, 10mM EDTA). Quality and quantity of DNA sample were examined through 0.8 per cent agarose gel electrophoresis and were further diluted to a uniform concentration of 50ng/ μ l. The extracted DNA samples, along with the diluted samples were stored at -40°C freezer (REMI quick freezer).

Primers :

Forward and reverse primer sequence and annealing temperature of 10 SSR markers of garlic were taken from (Cunha *et al.*, 2012). Primers used in this study are presented in Table D with sequence of forward

Table D: List of SSR markers used in genetic diversity of garlic			
Markers	Forward primers	Reverse primers	Annealing temperature ($^{\circ}$ C)
Asa 07	CTCGGAACCAACCAGCATA	CCCAAACAAGGTAGGTCAGC	58
Asa 08	TGATTGAAACGAATCCCACA	GGGGTTACCTGAACCTGTTA	56
Asa 10	TTGTTGTCTGCCATTTT	GATCTAAGCCGAGAGAAA	48
Asa 14	TCTATCTCGCTTCTCAGGGG	GCTGACAGAAAGTAGTCTTCC	48
Asa 16	CACGACTTTTCCCTCCCATTT	GCTAATGTTTCATGTCCCCAGT	48
Asa 17	TCCACGACACACACACACAC	ATGCAGAGAAATTTGGCATCC	56
Asa 18	TCAAGCTCCTCAAGTGTC	TCGGGATATGACAGCATTTG	45
Asa 24	TTGTTGTGCCGAGTTCATA	CAGCAATTTACCAAAGCCAAG	48
Asa 25	GCACTTCACTTTCCCCATTC	GGCGACGGTGAAGAGAGAG	51
Asa 31	CAGAGACTAGGGCGAATGG	ATGATGATGACGACGACGAG	50

primer and reverse primer, along with their annealing temperature.

PCR amplification through SSR primers :

Diluted DNA samples were subjected to PCR amplification, using the selected SSR primers in automated thermal cycler (Eppendorf master cycler model nexus). 94.72µl reaction volume contained 2µl (50ng) DNA, 11.20µl 10x PCR buffer [MgCl₂], 5.6µl 2mM dNTPs (Exeltris), 9.4µl mM forward primer, 9.4µl mM reverse primer, 1.12µl Taq DNA polymerase enzyme (3U/µl, exeltris) and 58.0µl autoclaved molecular biology water. Reaction condition was programmed as initial hold at 94°C for 3min followed by 30 cycles of denaturation at 94°C for 30s, annealing of primer at 58°C annealing temperature for 45s and extension at 72°C for 1min; 10 cycle at 94°C for 30s, at 2°C below the specific annealing temperature of each pair of primers for 45s and at 72°C for 1min and a final elongation step at 72°C for 10min followed by holding the samples at 4°C for 1min. On completion of reaction 6x purple dye was added and the amplification products were stored at -20°C freezer, till further analysis through agarose gel electrophoresis.

Agarose gel electrophoresis and primers:

Amplification products were subjected to agarose gel electrophoresis in 2% (W/V) agarose gel. For this 1g of agarose was weighed and poured into a conical flask containing 50ml of water and 1 ml of 50x TAE buffer. This was allowed to melt with frequent shaking, followed by cooling the gel to 65°C, 6µl of ethidium bromide (10mg/ml) was added, mixed by gentle swirling and then poured into gel tray to solidify. Amplification products were loaded and run for 1.30 hour at fixed voltage (80V). Banding pattern were visualized, photographed and scored on gel Doc. The frequency of SSR polymorphism was calculated on the basis of presence or absence of common bands where presence was denoted by 1 and absence was denoted by 0. Polymorphic information content (PIC) value was calculated by using the given formula (Chattopadhyay *et al.* 2008). $PIC = \frac{1}{n} \sum 2F(1-F)$

where, F = proportion of a particular allele among the genotypes, n = No. of alleles generated.

Statistical analysis:

The experimental data for various characters,

recorded in course of this investigation were subjected to statistical analysis using suitable technique for different characters. The observations recorded were subjected to statistical scrutiny. The results of the following parameters were analyzed.

Phenotypic variance:

Phenotypic variance was calculated by adding genotypic variance to error mean sum of square as suggested by Comstock and Robinson (1952);

$$V_{ph} = V_g + E$$

where, 'V_{ph}' is phenotypic variance, 'V_g' is genotypic variance, 'E' is error mean sum of square.

Genotypic variance :

Genotypic variance was calculated by subtracting the error mean sum of square from the treatment mean sum of square and dividing it by the number of replications as suggested by Comstock and Robinson (1952). $V_g = V - E/r$

where, 'V' is the treatment mean sum of square, 'E' is error mean sum of square, 'V_g' is genotypic variance, 'r' is number of replications. In the present study, the genotypic variance has been expressed as percentage of phenotypic variance. The error mean sum of square was taken as environmental variance *i.e.* $V_e = E$, it has been expressed as percentage of phenotypic variance in the present study.

Co-efficient of variation:

It is the ratio of standard deviation of a sample to its mean and expressed in percentage.

$$CV(\%) = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Phenotypic and genotypic co-efficients of variation were calculated by the method suggested by Burton and Devane (1953).

Phenotypic co-efficient of variation:

$$PCV = \frac{\text{Phenotypic standard deviation}}{\text{General mean}} \times 100$$

$$PCV = \frac{\sqrt{\frac{2}{p}}}{\bar{X}}$$

where, σ_p^2 = Phenotypic variance, \bar{X} = General mean.

Genotypic co-efficient of variation:

$$GCV = \frac{\text{Genotypic standard deviation}}{\text{General mean}} \times 100 \quad GCV = \sqrt{\frac{\sigma_g^2}{\bar{X}}}$$

where, σ_g^2 = Genotypic variance, \bar{X} = General mean
PCV and GCV were classified as low, moderate or high by Sivasubramanian and Menon (1973) as given below: (i) 0-10 per cent- Low (ii) 10-20 per cent- Moderate (iii) 20 per cent and above- High.

Correlation co-efficient analysis :

Correlation co-efficient is the mutual association between variables without implying any cause and effect relationship. Single correlation co-efficients were computed at genotypic and phenotypic levels between pair of characters adopting following formula given by Johnson *et al.* (1955).

Genotypic correlation between traits X and Y:

$$R_{xy}(g) = \frac{\sigma_{g(xy)}}{\sqrt{\sigma_g^2(x) \sigma_g^2(y)}}$$

where, $\sigma_{g(xy)}$ = Genotypic covariance between X and Y, $\sigma_g^2(x)$ = Genotypic variance for X, $\sigma_g^2(y)$ = Genotypic variance for Y.

Phenotypic correlation between traits X and Y:

$$R_{xy}(p) = \frac{\sigma_p(xy)}{\sqrt{\sigma_p^2(x) \sigma_p^2(y)}}$$

where, $\sigma_p(xy)$ = Phenotypic covariance between X and Y, $\sigma_p^2(x)$ = Phenotypic variance for X, $\sigma_p^2(y)$ = Phenotypic variance for Y.

Heritability:

Heritability in broad sense is the ratio of genotypic variance to the total variance and is calculated by the formula given by Lush (1940). $h^2 = \frac{\sigma_g^2}{\sigma_p^2}$

where, H = Heritability in broad sense, σ_g^2 = Genotypic variance, σ_p^2 = Phenotypic variance = $\sigma_g^2 + \sigma_e^2$ (MSE) The heritability per cent was categorized by Johnson *et al.* (1955) as:- (i) 0-30 per cent- Low (ii) 30-60 per cent - Moderate (iii) 60 per cent and above- High. Genetic advance is the improvement in mean genotypic value of selected plants over the parental population. The estimates of genetic advance were obtained by the

formula given by Johnson *et al.* (1955):- $GA = K \cdot \sigma_p \cdot H$
where, K= Constant selection differential at 5 per cent level intensity (2.06), σ_p = Phenotypic standard deviation, H= Heritability in broad sense. Genetic advance as percentage of mean was calculated by the following formula:

$$\text{Genetic advance as percentage of mean} = \left(\frac{GA}{\bar{X}} \right) \times 100$$

where, GA = Expected genetic advance, \bar{X} = General mean of the character in the population, The GA as per cent of mean was categorized by Johnson *et al.* (1955) as: (i) 0-10 per cent- Low (ii) 10-20 per cent- Moderate (iii) 20 per cent and above- High.

Path co-efficient analysis :

The path co-efficient analysis is simply the standardized partial regression co-efficient, which splits the correlation co-efficient into the measures of direct and indirect effects of independent variables on the dependent variables. The concept of path analysis was originally developed by Wright (1921), but this technique was firstly used for plant selection by Dewey and Lu (1959). Path co-efficient was estimated by solving the equation written here in matrix form, which is as follows:

$$(A)_{0 \times 1} = (B)_{0 \times 1} (C)_{0 \times 1}$$

where, A is column of correlation r_{ij} B is correlation matrix of r_{ij} and C is column vector of direct effects, p_{iy} Residual factor was calculated as follows: $P_{xy} = (1 - R^2)^{1/2}$

where $R^2 = \sum r_{ij} p_{iy} r_{ij}$ = Correlation co-efficient between i^{th} and y character p_{iy} = Direct effect of i^{th} character on y .

Genetic diversity analysis: D² statistics :

The concept of D² statistics for a measure of group distance based on multiple characters was developed by Mahalanobis in 1928. The estimation of D² values by formula: $D^2 = w^{ij}(x_i^{-1} - x_j^{-1})(x_i^{-1} - x_j^{-1})$ is very complicated since it needs inversion of matrix of high order when the number of character are large.

Computation of D² value and their significance:

For each combination, the mean deviation *i.e.* $Y_i^1 - Y_i^2$ is computed and the D² value is computed as sum of squares of these deviations. $D^2 = \sum (Y_i^1 - Y_i^2)^2$

where, $i = 1, 2, \dots, p$ -number of characters, Y_i^1 = Transformed uncorrelated mean of i^{th} character for genotype 1, Y_i^2 = Transformed uncorrelated mean of i^{th}

character for genotype 2. The significance D^2 values are tested against the table value of X^2 at p degree of freedom, where, p is total number of characters included in the study. If the calculated D^2 values is higher than the table X^2 values, it is considered as significant and *vice-versa*.

Grouping of genotypes into clusters:

The first step in grouping the genotypes into distinct clusters is to arrange the genotypes in order of their relative distances from each other. Ward is used for cluster formation. He suggested a general agglomerative hierarchical clustering procedure, where the criterion for choosing the pair of clusters to merge at each step is based on the optimal value of an objective function. At each step the pair of clusters with minimum between-cluster distance is merged. To implement this method, at each step find the pair of clusters that leads to minimum increase in total within-cluster variance after merging. This increase is a weighted squared distance between cluster centers. At the initial step, all clusters are singletons (clusters containing a single point). To apply a recursive algorithm under this objective function, the initial distance between individual objects must be squared Euclidean distance. The initial cluster distances in Ward's minimum variance method are therefore defined to be the squared Euclidean distance between points:

$$d_{ij} = d(\{X_i\}, \{X_j\}) = \|X_i - X_j\|^2$$

Intra and inter cluster distance :

The formula for the measure of average intra-cluster distance is $\frac{\sum D_i^2}{n}$ where $\sum D_i^2$ is the sum of distances between all possible combinations (n) of the genotypes included in a cluster. The procedure for calculating the inter-cluster distance is first to measure the distance between various combinations of clusters and divides by the product of the number of genotypes in the concerned cluster combinations. Average inter cluster distance

$$\text{between cluster } i \text{ and } j = \frac{\sum D_i^2}{n_i \times n_j}$$

where, $\sum D_i^2$ = Is the sum of distance between the genotypes in cluster i and cluster j , = Number of genotypes in cluster i . n_j = Number of genotypes in cluster j .

Cluster diagram :

With the help of D^2 values between (inter-cluster distance) and within (intra-cluster distance) clusters, a diagram showing the relationship between different genotypes can be drawn. Such a diagram is not exactly to the scale.

Contribution of individual characters towards total divergence:

In all the combination of genotypes, $\frac{n(n-1)}{2}$, each character is ranked on the basis of mean difference, *i.e.*, $d_i = Y_i^1 - Y_i^2$ value, rank 1 is given to the highest mean difference and rank p to the lowest mean difference where p is the total number of characters. Using these ranks, the following table was prepared to work out the per cent contribution of each character to the total divergence.

RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads and Table 1 to 3.

Morphological characterization :

The observations recorded in twenty seven garlic germplasm on twelve morphological traits *viz.*, plant height, number of leaves per plant, leaf length, breadth of leaves, neck thickness, polar diameter of bulb, equatorial diameter of bulb, bulb weight per plant, number of cloves per bulb, length of the clove, width of clove and clove weight were analyzed statistically for different genetic parameters and the results are presented under the following broad aspects of this study.

Analysis of variance :

Mean data for 12 characters were subjected to analysis of variance for the RBD. The mean sum of squares due to genotypes was significant for all the 12 characters under study which indicated that the genotypes included in the study were genetically diverse and considerable amount of variability were present in the genotypes. Hence, there is ample scope for getting promising hybrids in breeding programme for yield and quality characters.

Mean performance of all the characters:

The mean performances of the genotypes and

checks for all the characters under study have been presented in Table 2. In general a wide range of mean values within the genotypes have been found for the respective characters. Character wise mean performance of the genotypes are being discussed here under.

Growth characters :

Plant height :

Data pertaining to plant height in garlic revealed that the range for this character for genotype varied between 31.43 cm to 52.07 cm, respectively. The genotype IC 375119 was found to be taller while 453 found to be dwarf for plant height. The grand mean of this character was 40.48 cm.

Number of leaves per plant :

The data presented in Table 2 indicated that all genotypes showed wide range of variation with respect to number of leaves per plant. Cultivar BRG-10 produced maximum number of leaves followed by cultivar 644 and IC 375119. While the cultivar IC 375107 had the minimum number of leaves per plant. The grand mean value for number of leaves was 5.73.

Leaf length :

It is indicated from Table 2 that the leaf length different each other among the cultivars and maximum leaf length was recorded in 644 followed by IC 375119 and cultivar WG 7-1 had minimum leaf length of 25.20cm followed by RG 464. The range of leaf length was between 25.20cm to 47.96cm. The grand mean of this character was 36.49cm.

Breath of leaves:

It is clearly indicated in Table 2 that the cultivars exhibited difference in terms of breath of leaves and cultivar recorded maximum breath was in BRG-8. Whereas, minimum was recorded in 543 was observed. The grand mean for this character was 1.58 cm.

Neck thickness :

The neck thickness exhibited the range of 0.91cm and 1.37cm and it was maximum observed with 644 followed by WG 7-1 and minimum neck thickness was observed with 569. The grand mean value for this character was 1.17cm.

Polar diameter of bulb:

The range for polar diameter of bulb was 2.81cm to 3.93 cm and the grand mean for this character was 3.32 cm. The maximum polar diameter of bulb was noted with 644 followed by BRG-14 and minimum polar diameter of bulb was observed with cultivar 453.

Equatorial diameter of bulb:

The data given in Table 2 showed that the bulb diameter different among the germplasm and 453 exhibited the maximum bulb equatorial diameter followed by BRG-14. Whereas, the minimum equatorial bulb diameter was recorded with WG-7. The grand mean for equatorial bulb diameter was 3.30cm.

Clove length :

The data presented in Table 2 indicates that clove

Sources of variation	Degree of freedom	Mean squares											
		PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
Replication	2	1.69	0.68	0.38	0.02	0.001	0.01	0.01	0.06	0.01	0.18	0.00	0.30
Genotypes	26	76.10**	2.02**	90.24**	0.08**	0.048**	0.26**	0.13**	0.20**	0.02**	53.46**	0.04**	27.51**
Error	52	1.74	0.25	2.52	0.01	0.000	0.06	0.07	0.05	0.01	2.08	0.00	0.92
PH		Plant height (cm)					EDB			Equatorial diameter of bulb (cm)			
Le/P		Leaves per plant					LC			Length of clove (cm)			
Lle		Length of leaf (cm)					WdC			Width of clove (cm)			
BrLe		Breath of leaf (cm)					C/B			Cloves per bulb			
NTh		Neck thickness (cm)					AWC			Average weight of clove (g)			
PDB		Polar diameter of bulb (cm)					Y/P			Yield per plant (g)			

** indicate significance of value at P=0.01

length varies among the germplasm and the range for clove length was 2.17cm to 3.13cm with 644 showed maximum clove length followed by 4 whereas, WG 2 showed minimum clove length. The grand mean value for clove length was 2.51cm.

Width of clove :

It is evident from the data that variation for width of clove was among the germplasm. The maximum width was recorded with 569 followed by IC 375119 whereas, minimum was observed in cultivar 527. The grand mean for width of clove was 0.83cm.

Number of cloves per bulb:

Number of cloves per bulb also showed differences among the germplasm and the maximum number of cloves per bulb was observed in BRG-14 followed by 453. Whereas, minimum number of cloves per bulb was found in 569. The grand mean for number of cloves per bulb was 23.13.

Clove weight:

Clove weight exhibited difference among the germplasm. The maximum single clove weight was shown by 644. Whereas, minimum single clove weight

Table 2 : Mean performance of 27 genotypes of garlic for 12 characters

Sr. No.	Character/genotype	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
1.	4	38.47	5.30	35.43	1.70	0.93	3.81	3.49	3.05	0.84	21.00	0.66	15.72
2.	BRG-14	46.97	5.53	42.17	1.60	1.26	3.90	3.61	2.56	0.91	31.37	0.53	17.22
3.	G-232	44.27	5.27	40.10	1.61	1.02	3.38	3.36	2.53	0.87	25.47	0.61	16.53
4.	BRG-7	39.27	5.30	36.27	1.50	1.16	3.45	3.03	2.19	0.71	27.47	0.35	11.64
5.	BRG-13	37.87	5.87	34.23	1.60	1.16	3.01	3.16	2.28	0.80	20.43	0.54	12.55
6.	IC-345585	37.23	5.43	36.27	1.52	1.22	3.15	3.52	2.33	0.79	27.23	0.50	15.26
7.	453	51.13	5.10	43.43	1.47	1.28	2.88	3.65	2.41	0.77	29.30	0.45	17.39
8.	BRG-9	34.23	5.73	29.53	1.63	1.33	3.24	3.35	2.44	0.74	28.67	0.42	16.65
9.	644	44.67	7.63	47.97	1.77	1.37	3.93	3.43	3.13	0.89	24.00	0.91	26.19
10.	569	41.33	6.27	36.60	1.73	0.92	3.32	3.39	2.66	1.02	14.10	0.50	13.18
11.	WG-73	42.40	5.07	37.07	1.70	1.05	3.35	3.18	2.16	0.84	20.10	0.63	13.53
12.	WG-7	43.27	5.07	38.27	1.43	1.33	3.54	3.43	2.44	0.80	23.00	0.45	13.44
13.	RG-464	31.43	5.17	28.50	1.33	1.10	3.19	3.13	2.50	0.75	20.80	0.52	11.05
14.	IC-375107	36.93	5.07	32.23	1.60	1.27	3.30	3.41	2.75	0.79	24.20	0.50	15.24
15.	543	34.10	5.27	32.30	1.23	1.27	3.14	3.10	2.51	0.82	23.33	0.45	11.21
16.	WG-2	39.83	5.20	35.50	1.23	1.34	3.03	3.33	2.11	0.84	25.53	0.40	13.24
17.	650	39.70	5.20	36.30	1.70	1.30	3.45	3.42	2.47	0.72	22.43	0.52	15.68
18.	BRG-10	45.10	8.10	41.30	1.75	1.12	3.48	3.45	2.53	0.76	29.00	0.53	17.64
19.	RAU-G5	40.17	6.13	35.33	1.73	1.06	3.28	3.06	2.96	0.96	15.43	0.65	14.82
20.	IC-375119	52.07	7.63	47.63	1.77	1.11	2.92	3.20	2.52	1.01	22.23	0.53	13.69
21.	G1	45.13	6.04	43.83	1.70	1.17	3.66	3.56	2.13	0.91	20.33	0.44	14.30
22.	BRG-3	39.10	5.40	31.77	1.50	1.27	3.39	3.33	2.68	0.76	25.90	0.56	16.08
23.	IC-290440	42.63	6.10	39.20	1.73	1.03	3.59	3.54	2.35	0.87	21.43	0.68	13.39
24.	527	35.17	5.63	32.80	1.73	1.07	2.90	3.07	2.47	0.67	22.67	0.46	11.66
25.	BRG-8	39.40	5.50	34.07	1.80	1.13	3.14	3.05	2.76	0.78	23.43	0.42	13.24
26.	M-175	35.17	5.47	32.13	1.37	1.07	3.30	3.12	2.46	0.79	18.67	0.64	12.43
27.	WG-7-1	35.97	5.33	25.20	1.43	1.19	2.93	2.86	2.42	0.83	17.10	0.59	12.18
	Mean	40.48	5.73	36.49	1.58	1.17	3.32	3.3	2.51	0.83	23.13	0.53	14.64
	C.V.	3.25	8.72	4.35	6.22	1.68	7.38	7.91	8.65	8.71	6.23	9.69	6.54
	S.E.±	0.76	0.29	0.92	0.06	0.01	0.14	0.15	0.13	0.04	0.83	0.03	0.55
	C.D. (P=0.01)	2.88	1.09	3.46	0.22	0.04	0.53	0.57	0.47	0.16	3.15	0.11	2.09

was shown by cultivar BRG-7. The grand mean value for the single clove weight was 0.53g.

Yield per plant:

The data pertaining to bulb weight per plant among the cultivars in Table 2 indicates that the bulb weight per plant had the range of 11.05g to 26.19g. The maximum bulb weight was observed in 644 followed by BRG-10. Whereas, the minimum bulb weight was observed with cultivar RG 464. The grand mean for this character is 14.64g.

Genotypic and phenotypic variance :

Maximum genotypic and phenotypic variance was observed for weight of clove, yield per plant, number of cloves per bulb, leaf length, leaves per plant and plant height. Low levels of genotypic and phenotypic variances were shown by polar and equatorial diameter of bulb,

clove length and breadth of leaves.

Genotypic and phenotypic co-efficient of variation:

The results revealed that the phenotypic co-efficient of variation were higher than those of genotypic co-efficient of variation. The highest genotypic co-efficient of variation was observed for average clove weight followed by yield per plant. However, clove per bulb, leaf length, leaves per plant, plant height and width of clove exhibited the moderate genotypic co-efficient of variation.

Heritability in broad sense (h^2_{bs}):

The data presented in Table 3 revealed that the broad sense heritability ranged from 23 per cent to 98 per cent. When compared, all the character like width of clove, plant height, leaf length, yield per plant clove per bulb, average weight of clove, breath of leaves and

Table 3: Mean, range, co-efficient of variation, heritability, genetic advance and genetic advance as per cent of mean

Characters	General mean	Range	Co-efficient of variation			Heritability (h^2_{bs}) %	Genetic advancement	Gen. adv as % of mean
			GCV	PCV	ECV			
PH	40.48	31.43-52.07	12.30	12.72	3.25	93	12.71	31.39
Le/P	5.73	5.07-8.10	13.39	15.98	8.72	70	1.70	29.63
LLe	36.50	25.20-48.00	14.82	15.44	4.35	92	13.70	37.53
BrLe	1.59	1.23-1.80	9.84	11.64	6.22	71	0.35	21.95
NTh	1.17	0.91-1.37	9.21	12.67	8.71	53	0.15	17.66
PDB	3.32	2.81-3.93	7.73	10.69	7.38	52	0.49	14.77
EDB	3.30	2.86-3.65	4.31	9.01	7.91	23	0.18	5.45
LC	2.51	2.17-3.13	8.92	12.42	8.65	52	0.42	16.91
WdC	0.82	0.67-1.01	9.21	12.67	8.71	53	0.15	17.66
C/B	23.14	14.1-31.37	17.89	18.94	6.23	89	10.32	44.60

Table 4 : Estimates of phenotypic correlation co-efficient between different characters in garlic

Characters	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
PH	1.000	0.404**	0.855**	0.365**	0.019	0.172	0.368**	0.033	0.426**	0.253	0.121	0.436**
Le/P		1.000	0.517**	0.489**	-0.092	0.153	0.079	0.249	0.356**	0.023	0.349*	0.411**
LLe			1.000	0.407**	0.049	0.341*	0.394**	0.055	0.385**	0.250	0.217	0.554**
BrLe				1.000	-0.330*	0.201	0.150	0.266	0.256	-0.099	0.284	0.335*
NTh					1.000	-0.002	0.156	-0.104	-0.257	0.524**	-0.181	0.346*
PDB						1.000	0.301*	0.226	0.114	0.079	0.308*	0.468**
EDB							1.000	0.020	0.170	0.404**	0.096	0.440**
LC								1.000	0.193	-0.099	0.462**	0.381**
WdC									1.000	-0.312	0.300*	0.112
C/B										1.000	-0.264	0.396**
AWC											1.000	0.531**
Y/P												1.000

* and ** indicate significance of values at P=0.05 and 0.01, respectively

showed high heritability.

Genetic advance:

The data has shown in Table 3 reveals that the genetic advancement was observed moderate for length of leaves, plant height, cloves per bulb. Low was reported in Yield per plant, number of leaves per plant, polar diameter of bulb, length of clove, breath of leaves, width of clove, clove weight and equatorial diameter of bulb.

Genetic advance in percentage of mean:

The data shown in Table 3 reveals that the genetic advance expressed as percentage of mean was observed high for eight characters. The highest value for genetic

advance as percentage of mean was shown by yield per plant followed by average weight of clove, cloves per bulb, length of clove, plant height, leaves per plant width of clove, breath of leaves.

Association analysis:

The phenotypic and genotypic correlation co-efficient for all characters is presented in the Table 4 and 5. The estimation of correlation co-efficient revealed that, genotypic correlation was higher in magnitude than the corresponding phenotypic correlation co-efficients.

Phenotypic correlation for morphological and yield attributing traits:

The statistical data relating to phenotypic correlation co-

Table 5 : Estimates genotypic correlation co-efficient between different characters in garlic

Characters	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
PH	1.000	0.478**	0.912**	0.411**	0.029	0.247	0.748**	-0.017	0.562**	0.261	0.136	0.463**
Le/P		1.000	0.608**	0.568**	-0.107	0.210	0.161	0.326*	0.433**	-0.020	0.389**	0.496**
LLe			1.000	0.459**	0.049	0.439**	0.851**	0.108	0.546**	0.278	0.277	0.594**
BrLe				1.000	-0.412**	0.394**	0.238	0.464**	0.304*	-0.152	0.340*	0.404**
NTh					1.000	0.002	0.271	-0.133	-0.367**	0.551**	-0.193	0.362**
PDB						1.000	0.837**	0.472**	0.288*	0.168	0.564**	0.620**
EDB							1.000	0.072	0.172	0.568**	0.076	0.820**
LC								1.000	0.293*	-0.192	0.648**	0.561**
WdC									1.000	-0.522**	0.388**	0.150
C/B										1.000	-0.329*	0.416**
AWC											1.000	0.604**
Y/P												1.000

* and ** indicate significance of values at P=0.05 and 0.01, respectively

Table 6 : Direct and Indirect effect of different characters on bulb weight/plant at phenotypic level

Characters	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
PH	0.042	0.017	0.036	0.015	0.001	0.007	0.015	0.001	0.018	0.011	0.005	0.436
Le/P	0.019	0.046	0.024	0.022	-0.004	0.007	0.004	0.011	0.016	0.001	0.016	0.411
LLe	0.160	0.097	0.187	0.076	0.009	0.064	0.074	0.010	0.072	0.047	0.041	0.554
BrLe	0.059	0.079	0.066	0.162	-0.054	0.033	0.024	0.043	0.042	-0.016	0.046	0.335
NTh	0.006	-0.030	0.016	-0.109	0.331	-0.001	0.052	-0.034	-0.085	0.173	-0.060	0.346
PDB	0.025	0.023	0.050	0.030	0.000	0.148	0.045	0.033	0.017	0.012	0.046	0.468
EDB	0.035	0.008	0.038	0.014	0.015	0.029	0.096	0.002	0.016	0.039	0.009	0.440
LC	0.005	0.036	0.008	0.039	-0.015	0.033	0.003	0.145	0.028	-0.014	0.067	0.381
WdC	-0.030	-0.025	-0.028	-0.018	0.018	-0.008	-0.012	-0.014	-0.072	0.022	-0.021	0.112
C/B	0.061	0.006	0.060	-0.024	0.125	0.019	0.097	-0.024	-0.075	0.239	-0.063	0.396
AWC	0.054	0.156	0.097	0.127	-0.081	0.137	0.043	0.206	0.134	-0.117	0.446	0.531

Residual effect= 0.4472

efficient has been presented in Table 4.

Genotypic correlation for morphological and yield attributing traits:

The analysis of data related to correlation co-efficient revealed that in all the characters under study, the genotypic correlation co-efficients were higher than their corresponding phenotypic correlation co-efficients as has been presented in Table 5.

Path analysis:

Partitioning of the correlation co-efficient of the various characters under study was done with the help

of path co-efficient analysis to express the direct and indirect effect of all these characters on yield per plant. The results obtained at genotypic and phenotypic level are presented in Table 5.

Phenotypic path analysis in relation to total yield per plant:

The statistics relating to the path co-efficient analysis representing direct and indirect contribution towards yield have been presented in Table 8 along with residual effect.

Genotypic path analysis in relation to yield per plant:

The statistics relating to the path co-efficient analysis

Table 7 : Direct and indirect effect of different characters on bulb weight/plant at genotypic level												
Characters	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
PH	0.985	0.471	0.898	0.405	0.029	0.244	0.737	-0.017	0.554	0.257	0.133	0.463
Le/P	0.153	0.321	0.195	0.182	-0.034	0.067	0.052	0.105	0.139	-0.007	0.125	0.496
LLe	0.067	0.045	0.074	0.034	0.004	0.032	0.063	0.008	0.040	0.020	0.020	0.594
BrLe	-0.162	-0.225	-0.182	-0.395	0.163	-0.156	-0.094	-0.183	-0.120	0.060	-0.134	0.404
NTh	0.006	-0.022	0.010	-0.084	0.204	0.000	0.055	-0.027	-0.075	0.112	-0.039	0.362
PDB	0.139	0.118	0.247	0.222	0.001	0.563	0.471	0.266	0.162	0.094	0.317	0.620
EDB	-0.072	-0.015	-0.081	-0.023	-0.026	-0.080	-0.096	-0.007	-0.017	-0.054	-0.007	0.820
LC	-0.010	0.194	0.064	0.276	-0.079	0.280	0.043	0.594	0.174	-0.114	0.385	0.561
WdC	-0.532	-0.409	-0.517	-0.288	0.348	-0.272	-0.163	-0.277	-0.946	0.494	-0.367	0.150
C/B	-0.114	0.009	-0.122	0.067	-0.242	-0.074	-0.249	0.084	0.229	-0.439	0.144	0.416
AWC	0.004	0.010	0.007	0.009	-0.005	0.015	0.002	0.017	0.010	-0.009	0.026	0.604

Residual effect= 0.3624

Table 8 : Clustering pattern of 27 genotypes of garlic on the basis of genetic divergence		
Cluster number	No. of genotypes	Genotypes included
I	4	BRG-14, 453, WG 7, WG 2.
II	2	IC-375119, G1.
III	8	BRG-7, IC 345585, BRG-10, IC-375107, BRG-3, 650, BRG-9, 543.
IV	1	644
V	6	BRG-13, WG 7-1, RG- 464, M-175, 527, BRG-8.
VI	6	569, RAU-G5, 4, WG 73, IC-290440, G-282

Table 9 : Average clusters distance of 27 genotypes of garlic						
Cluster No.	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster
1 Cluster	86.12	178.22	149.98	334.01	311.85	449.55
2 Cluster		60.00	207.79	459.54	228.67	214.12
3 Cluster			84.91	445.30	134.45	280.97
4 Cluster				0.00	681.87	752.91
5 Cluster					65.52	145.09
6 Cluster						92.38

representing direct and indirect contribution towards total yield have been presented in Table 9 along with residual effect and investigation of the data shows that plant height exerted maximum direct positive effect on total yield per plant.

Mahalanobis D² analysis : Clustering patterns :

On the basis of Mahalanobis D² values, all the 27 genotypes were grouped under study were grouped into six clusters. Cluster III contained maximum number of genotypes (8) comprising BRG-7, IC 345585, BRG-10, IC-375107, BRG-3, 650, BRG-9, 543; followed by cluster V containing 6 genotypes viz., BRG-13, WG 7-1, RG-464, M-175, 527, BRG-8 also, cluster VI comprising (6) genotypes namely 569, RAU-G5, WG 73, IC-290440, G-282. Cluster I containing (4) genotypes viz., BRG-14, 453, 7, WG 2. Cluster II comprising of (2) genotypes namely IC-375119, G1.

D² analysis (Rank method): Contribution of various characters towards total genetic divergence :

Ranking character wise D² values and adding the ranks for each character for all the entries identified the variables, which contributed towards the divergence. Neck thickness contributed maximum towards total divergence and this was followed plant height, cloves per bulb, length of leaves and average weight of clove have a same amount of contribution, yield per plant, leaves per plant, polar diameter of bulb, equatorial diameter of bulb, length clove and width of clove have zero contribution.

Allelic diversity analysis:

The 27 genotypes were screened with 10 SSR markers. Amplification profile of 7 SSR markers, allelic diversity of the genotypes and relative study of the amplification profiles (resolved on 2% agarose gel) are described below.

Amplification profile and allelic diversity analysis of SSR marker Asa 07 :

As shown in Fig. 1 the SSR marker Asa 07 generated a total of five different alleles range from 146bp to 300bp. Allele 4 was found to be most frequent among the genotypes and hence considered as the high frequency allele with a frequency of 0.44. This high frequency allele was found in the genotypes *i.e.* BRG-14, G 282, BRG-7, BRG-13, WG 73, WG 7, 543, RAU-G5, BRG-3, BRG-8, M-175 and WG 7-1. Allele 5 was found in genotypes *i.e.* IC-375119, G1, IC-290440 AND 527. Allele 3 (247 bp) was found in genotypes *i.e.* 4, IC 345585, 453, 569, RG-464, IC-375107, and WG 2. Allele 2 (220 bp) was found in genotypes *i.e.* BRG-9, 650 and BRG-8. One rare allele was recorded in genotype 644 (146 bp). Polymorphism Information Content value of SSR marker Asa 07 was found to be 0.280.

Amplification profile and allelic diversity analysis of SSR marker Asa 08:

As shown in Fig. 2, SSR marker Asa 08 was observed to generated four alleles range from 120bp to 266 bp. Alleles 1 was found to be most frequent among the genotypes and hence, considered as the high

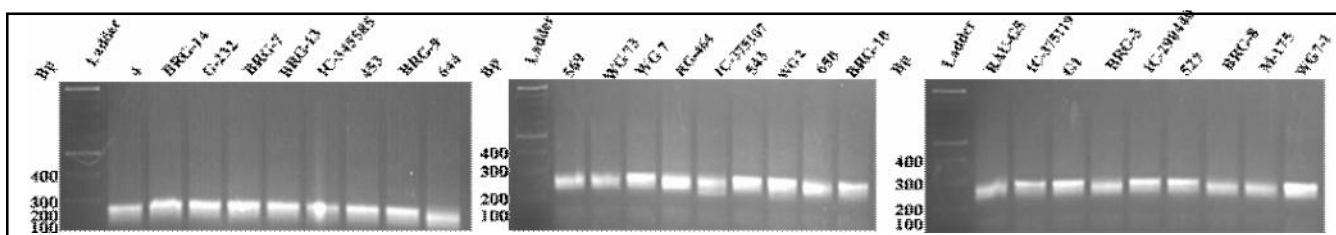


Fig. 1 : Ethidium bromide stained 2 per cent agarose gel showing amplification profile of SSR marker Asa 07

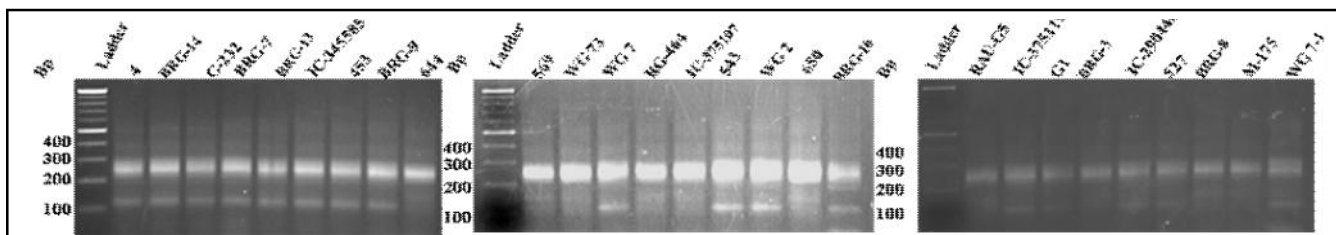


Fig. 2 : Ethidium bromide stained 2 per cent agarose gel showing amplification profile of SSR marker Asa 08

frequency allele with a frequency of 0.62. This high frequency allele is found in genotypes 4, BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, BRG-9, WG 7, 543, WG 2, BRG-8, IC-375119, G1, IC-290440, 527 and WG 7-1. Allele 3 was found in genotype *i.e.* 4, BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, 569, WG 73, IC-375107, 650, BRG-8, RAU-G5, IC-375119, G1 and BRG-3. Alleles 4 was found in genotype WG 7, RG-464, 543, WG 2, IC-290440, 527, BRG-8, M-175 and WG 7-1. Allele 2 found in two genotype BRG-9 and 644. PIC value of Asa 08 was found to be 0.306.

Amplification profile and allelic diversity analysis of SSR marker Asa 10:

Fig. 3 reveals that SSR marker Asa 10 generated allele in the present study, of 225bp and 246bp. 27 genotypes. Alleles were found in all the genotypes except two genotypes. PIC value of SSR marker Asa 10 was 0.137.

Amplification profile and allelic diversity analysis

of SSR marker Asa 14:

As shown in Fig. 4 SSR marker Asa 14 generated four alleles from 120bp to 360bp. Allele 3 was found to be most frequent among the genotypes and hence considered as the high frequency allele with a frequency of 0.66. This high frequency allele was found in the genotypes *i.e.* 4, BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, 644, WG 7, 543, WG 2, BRG-8, IC-375119, G1, 527, BRG-8, M-175 and WG 7-1. Alleles 4 was found in genotypes 644, WG 73, RG 464, 650 and BRG-8. Allele 2 was found in genotypes *i.e.* BRG-9, 644, WG 73, RG 464, IC-375107, 650 and BRG-3. Alleles 1 was found in genotypes 644, 569, WG 73, WG 7, RG-464, IC-375107, 650, RAU-G5, BRG-3, BRG-8 and M-175. PIC value of SSR marker Asa 14 was found to be 0.403.

Amplification profile and allelic diversity analysis of SSR marker Asa 16:

As shown in Fig. 5 Asa 16 generated five alleles from 148bp to 505bp. Alleles 2 was found to be most frequent among the genotypes and hence considered as

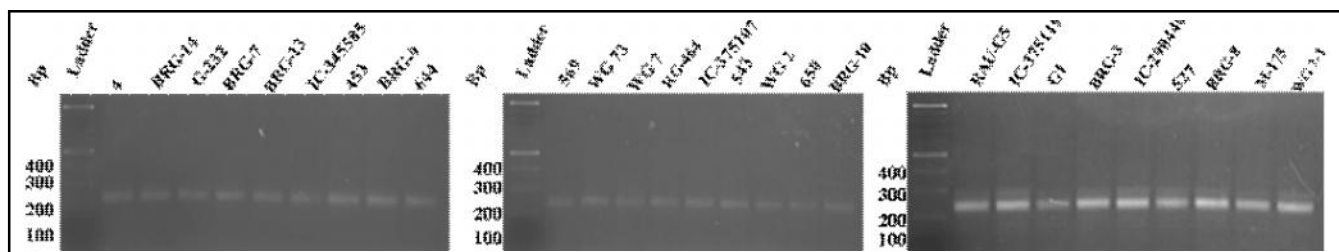


Fig. 3 : Ethidium bromide stained 2 per cent agarose gel showing amplification profile of SSR marker Asa 10

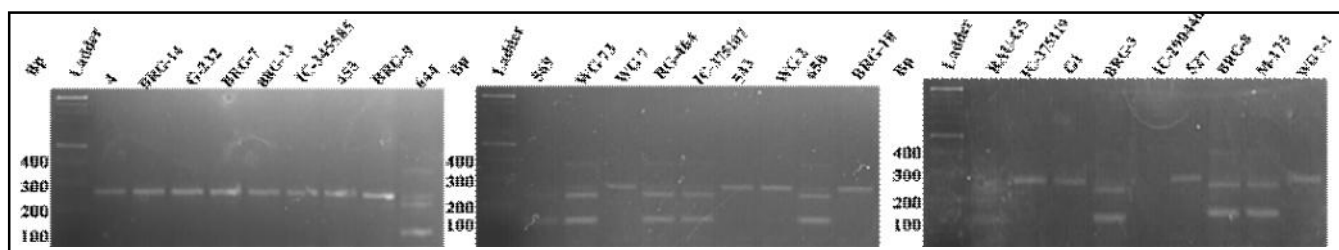


Fig. 4 : Ethidium bromide stained 2 per cent agarose gel showing amplification profile of SSR marker Asa 14

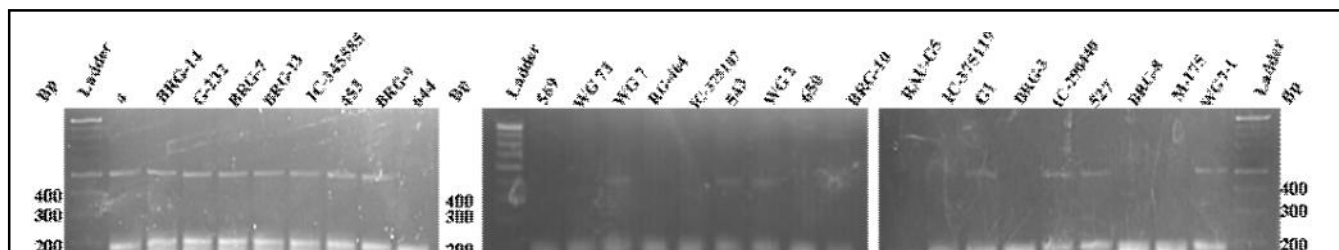


Fig. 5 : Ethidium bromide stained 2 per cent agarose gel showing amplification of Asa 16

the high frequency allele with a frequency of 0.629. This i.e. BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, 650, BRG-8, RAU-G5, IC-375119, G1, BRG-3, IC-290440, 527, BRG-8, M-175 and WG 7-1. Alleles 5 (505 bp) was

found in genotypes i.e. 4, BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, BRG-9, WG 7, 543, WG 2, G1, IC-290440, 527 and WG 7-1. Allele 4 was found in genotypes i.e. BRG-14, G-282, BRG-7, BRG-13, IC 345585, 453, BRG-9, 7, RG-464, IC-375107, 543, WG 2, 527 and BRG-

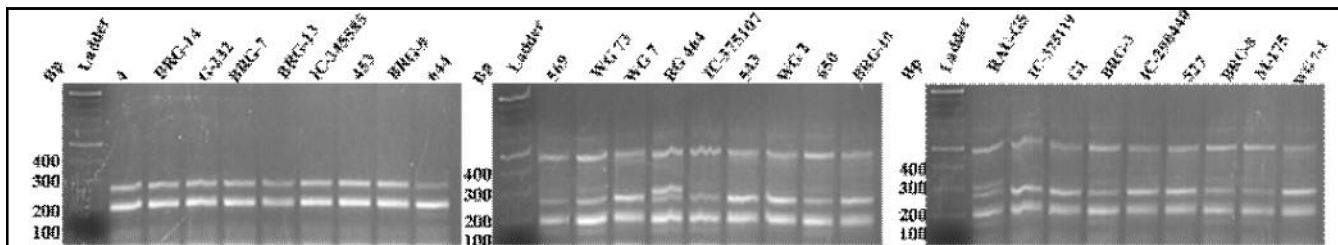


Fig. 6 : Ethidium bromide stained 2 per cent agarose gel showing amplification of Asa 18

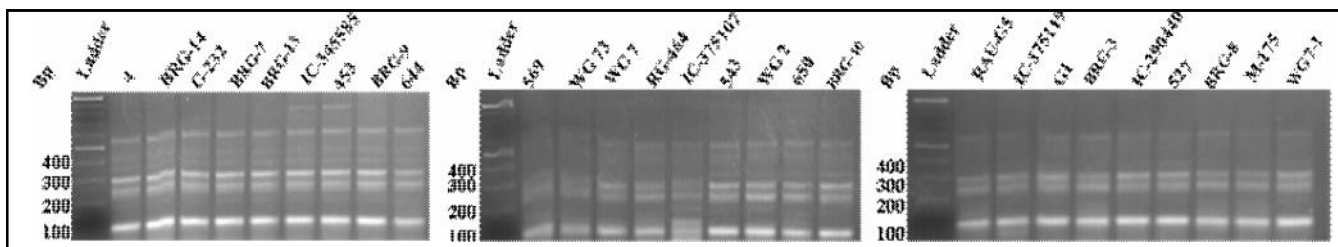


Fig. 7 : Ethidium bromide stained 2 per cent agarose gel showing amplification profile of SSR marker Asa 31

Table 10 : Cluster wise mean values of 12 characters in garlic

Cluster Number	Characters											
	PH	Le/P	LLe	BrLe	NTh	PDB	EDB	LC	WdC	C/B	AWC	Y/P
1 Cluster	45.30	5.22	39.84	1.43	1.30	3.34	3.51	2.38	0.83	27.30	0.46	15.32
2 Cluster	48.60	6.84	45.73	1.73	1.14	3.29	3.38	2.33	0.96	21.28	0.48	14.00
3 Cluster	38.21	5.69	34.50	1.55	1.24	3.32	3.33	2.49	0.76	26.03	0.48	14.92
4 Cluster	44.67	7.63	47.97	1.77	1.37	3.93	3.43	3.13	0.89	24.00	0.91	26.19
5 Cluster	35.83	5.49	31.16	1.54	1.12	3.08	3.06	2.48	0.77	20.52	0.53	12.19
6 Cluster	41.54	5.69	37.29	1.70	1.00	3.46	3.34	2.62	0.90	19.59	0.62	14.53

Table 11 : Contribution of various characters towards total genetic divergence

Sr. No.	Source	Times ranked 1 st	Contribution %
1.	Plant height (cm)	61	17.38
2.	Leaves per plant	3	0.85
3.	Length of leaf (cm)	17	4.84
4.	Breath of leaf (cm)	4	1.14
5.	Neck thickness (cm)	201	57.26
6.	Polar diameter of bulb (cm)	1	0.28
7.	Equatorial diameter of bulb (cm)	0.01	0.00
8.	Length of clove (cm)	0.01	0.00
9.	Width of clove (cm)	0.01	0.00
10.	Cloves/ bulb	39	11.11
11.	Average weight of clove (g)	17	4.84
12.	Yield per plant (g)	8	2.28

8. Alleles 3 were found in genotypes 4, 644, 569, WG 73, BRG-3 IC-290440, M-175 and WG 7-1. Alleles 1 were found in genotypes 4, 644 and RAU-G5. PIC value of SSR marker Asa 14 was found to be 0.415 (Table 13).

Amplification profile and allelic diversity analysis of SSR marker Asa 18:

As shown in Fig. 6, SSR marker Asa 18 generated four alleles from 220bp to 500bp. Alleles 1 and 2 was found in all the genotypes and hence, considered as the

Table 12 : Eight qualitative characters of 27 genotypes of garlic

Genotypes	Plant growth habit	Colour of leaves	Bulbils	Bulb wrap texture	Bulb skin colour	Clove skin colour	Clove flesh colour	Pungency
4	S	LG	Absence	thick	creamy white	White	White	Pungent
BRG-14	E	G	Presence	very thin	creamy white	white pinkish	yellowish	Pungent
G-232	S	LG	Absence	very thin	white creamy	White	yellowish	Very pungent
BRG-7	S	LG	Absence	very thin	cram white	White	yellowish	Pungent
BRG-13	S	LG	Absence	thin	white	White	yellowish	Medium
IC-345585	S	LG	Presence	very thin	silver white	white pinkish	yellowish	Pungent
453	S	LG	Absence	very thin	creamy white	white pinkish	yellowish	Medium
BRG-9	E	DG	Absence	thick	creamy white	white pinkish	yellowish	Pungent
644	E	DG	Absence	very thin	pink	White	White	Medium
569	E	G	Absence	medium	pink	White	White	Pungent
WG-73	E	DG	Presence	medium	creamy white	white pinkish	yellowish	Medium
WG-7	S	LG	Presence	medium	creamy white	white pinkish	yellowish	Medium
RG-464	E	G	Absence	medium	creamy white	white pinkish	yellowish	Pungent
IC-375107	E	G	Absence	very thin	silver white	white pinkish	yellowish	Medium
543	S	LG	Absence	very thin	creamy white	White	White	Pungent
WG-2	S	LG	Presence	medium	creamy white	White	White	Medium
650	E	DG	Presence	medium	creamy white	White	White	Medium
BRG-10	S	G	Absence	medium	silver white	White	White	Very pungent
RAU-G5	E	G	Absence	medium	silver white	White	White	Very pungent
IC-375119	S	DG	Presence	thin	creamy white	white pinkish	yellowish	Medium
G1	S	LG	Absence	thick	creamy	white pinkish	yellowish	Medium
BRG-3	E	G	Absence	very thin	creamy white	White	White	Medium
IC-290440	S	LG	Presence	very thin	creamy white	White	White	Very pungent
527	E	DG	Absence	thick	silver white	White	White	Pungent
BRG-8	S	DG	Absence	thick	creamy white	White	White	Medium
M-175	E	G	Absence	thick	silver white	White	White	Medium
WG-7-1	S	LG	Absence	thick	creamy white	White	White	Medium

Note:-S = Spreadubg E = Erect LG= Light green G = Green DG= Dark green

Table 13 : Features of 7 SSR markers

SSR	Number of alleles	Size range (bp)	Highest frequency alleles		PIC
			Size (bp)	Frequency	
Asa 07	5	146-300	270	0.5694	0.2798
Asa 08	4	120-266	120	0.6296	0.3059
Asa 10	2	225-246	246	0.9259	0.1372
Asa 14	4	120-360	260	0.6667	0.4033
Asa 16	5	148-505	170	0.6296	0.4148
Asa 18	4	220-500	274,220	1	0.1454
Asa 31	7	120-875	310,260,120	1	0.1513

high frequency alleles. Allele 4 was found in the genotypes 569, WG 73, WG 7, RG- 464, IC-375107, 543, WG 2, 650, BRG-8, RAU-G5, IC-375119, G1, BRG-3, IC-290440, 527, BRG-8, M-175 and WG 7-1. Allele 3 found in RG 464 and RAU-G5. Alleles 220 and 274 bp was found in all the genotypes. PIC value of SSR marker Asa 18 was found to be 0.145 (Table 13).

Amplification profile and allelic diversity analysis of Asa 31 :

As shown in Fig. 7, Asa 31 generated seven alleles from 120 bp to 875bp. Allele 1,3 and 4 was found in all the genotypes and hence, considered as the high frequency alleles. Allele 7 was found among the genotypes IC 345585 and 453. Allele 6 was found in the genotypes G-282, BRG-7, BRG-13, IC 345585, 453, BRG-9 and 644. Allele 5 was present in genotype 4, WG 7, RG- 464, IC-375107, 543, WG 2, 650, BRG-8, RAU-G5, IC-375119, G1, BRG-3, IC-290440, 527, BRG-8, M-175 and WG 7-1. Allele 2 was present in only one genotype. PIC value of SSR marker Asa 31 was found to be 0.151. The results found were also more or less similar to the findings of Agrawal and Tiwari (2009); Cunha *et al.* (2012); Jabeen *et al.* (2010); Patil *et al.* (2013); Sengupta and Dwivedi (2007); Singh *et al.* (2014); Singh *et al.* (2012); Singh *et al.* (2013) and Vatsyayan (2013).

Conclusion :

The experimental data were subjected to statistical analysis for elucidating the information on genetic variation existing for different components of growth and yield. The genetic variability was assessed using the parameters like genotypic and phenotypic variance, genotypic co-efficient variation and phenotypic co-efficient of variation, heritability and genetic advance. The inter character correlation and path co-efficient analysis, D² analysis and molecular analysis were also carried out to know the relationship among various growth and yield components. Analysis of variance reveals highly significant difference among the accessions for all the quantitative and qualitative characters studied. Genotypic and phenotypic co-efficients of variation were high for average weight of clove, yield per plant, cloves per bulb and plant height. Width of clove, plant height and length of leaves, yield per plant, cloves per bulb, average weight of clove exhibited high heritability coupled with high genetic

advance as per cent of mean. These characters are governed by additive gene effects. It was also concluded that selection on the basis of these characters will be more useful for the improvement of this crop towards attaining higher yield. Correlation co-efficient studies showed that phenotypic correlation co-efficients are higher than genotypic correlation which indicates the inherent association among the characters dependent of environment influence. Yield per plant had a positive and highly significant correlation with plant height, length of leaves, average weight of clove, equatorial diameter, polar diameter and clove/bulb which implies that these characters were contributing to bulb weight and bulb yield/plant. All the combination of traits should be considered while selecting for high yielding genotypes. Improvement of bulb weight per plant may be achieved by exercising direct selection of plant height, length of leaves, weight of cloves, equatorial diameter of bulb, clove weight, number of cloves per bulb, polar diameter of bulb and clove length as these exhibited significant positive direct effect on bulb weight per plant coupled with high heritability and high genetic advance as per cent of mean. Characters had correlation co-efficient value at par with their direct effect on bulb yield per hectare. In Mahalanobis D² analysis, On the basis of Mahalanobis D² values, all the 27 genotypes were grouped under study were grouped into six clusters. Cluster III (8), cluster V (6) and cluster VI (6) contained maximum number of genotypes and Cluster I (4) and cluster II (2) comprising minimum number of genotypes and cluster IV contained single genotype. The grouping pattern of the genotypes suggested no parallelisms between genetic divergence and geographical distribution of genotypes. The intra-cluster was maximum in cluster VI reveals maximum genetic diversity followed by cluster I and clusters III whereas maximum inter-cluster generalized distance was between cluster IV and cluster VI indicates maximum divergence followed by cluster IV and V. It confers that selection of genotypes based upon large cluster distance from all the clusters may lead to favorable broad spectrum genetic variability for bulb yield improvement. The Cluster II had highest mean values for plant height, width of clove and genotypes in cluster IV had highest mean values of leaves per plant, length of leave, breath of leave, neck thickness, polar diameter, length of clove average weight of clove and yield per plant. Cluster mean values of I had highest for equatorial diameter and cloves per bulb. Ranking character

wise D^2 values and adding the ranks for each character for all the entries identified the variables, which contributed towards the divergence. Neck thickness contributed maximum towards total divergence and this was followed plant height, cloves per bulb, length of leaves and average weight of clove have a same amount of contribution, yield per plant, leaves per plant, polar diameter of bulb, equatorial diameter of bulb, length clove and width of clove have zero contribution. On the basis of result and discussion made so far, it may be concluded that plant height, length of leaf, width of clove and average weight of clove can be put to direct selection pressure for high yield in garlic because these characters exhibited high GCV, PCV, heritability and genetic advance as percent of mean besides exerting direct effect on yield. This needs to be considered carefully at the time of framing a breeding strategy for selection of high yielding varieties. Based on SSR marker assay, 27 garlic genotypes were analyzed. PIC values ranged from 0.145 to 0.415 with an average of 0.019. It may be concluded that Primer Asa-14 and Asa 16 can be used for genetic diversity analysis in garlic.

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