Original Research Article   https://doi.org/10.20546/ijcmas.2018.701.349

Genetic Divergence Studies for Bulbing and Related Traits in Garlic Germplasm during *Kharif* Season

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A B S T R A C T

Garlic (*Allium sativum*) is second important allium crop grown in India after onion. It spite of exclusively propagated by vegetative method, crop shows wide variation in agro-morphological traits. For developing varieties with good yield and other favourable traits use of breeding techniques in an option. But in garlic, inability of formation of seed becomes barrier for using introgression breeding techniques. This limits the genetic enhancement of crop, however clonal selection narrow down the gene pool of crop. Further garlic enjoys countywide distribution and worldwide dynamic importance. However in India its growth habit restricted to *Rabi* season only. ICAR-DOGR serve as National Active Germplasm Site (NAGS) for garlic, where 700 garlic germplasm being maintained, collected from different states of India. For checking agro-climatic adaptability of garlic genetic resource present experiment was laid out. Here, total 625 garlic accessions were evaluated during kharif season for nine quantitative and five qualitative traits in two replications for two years. In pooled analysis only 68 bulb forming accessions were selected for further studies. Average values of different traits were taken for secondary analysis as non-significant difference found between years to accessions interaction. Cluster analysis (Ward 1963 method) based on fourteen agro-morphological traits sorted 68 genotypes into five main groups as cluster 1 (11 accessions), cluster 2 (7 accessions), cluster 3 (3 accessions), cluster 4 (33 accessions), cluster 5 (14). Dendrogram based on hierarchal clustering grouped the genotypes based on their morphological traits, however the grouping of genotypes did not correspond with their geographic origin. Characters like marketable yield, polar diameter, equatorial diameter, average bulb weight, number of cloves per bulb and TSS were found to be major traits in distinguishing major cluster of garlic genotype at phenotypic level. Highest genetic distance was reported between accession 202 and DOGR-499 (14.44), however accession 695 and 14 found to be most similar with minimum genetic distance i.e. 0.95. Principal component analysis revealed that first five component contribute to maximum 95% of variability. This study is first report that covers characterization and adaptation of garlic genotypes during *kharif* season.

Introduction

Garlic (*Allium sativum* L.) is a diploid (2n = 16) bulbous crop grown worldwide for culinary and medicinal purpose. Its native home and existence as a truly wild species, is not yet cleared and pretend to be originated at Central Asia as discussed by Hong and Etoh,

1996. According to Vavilov (1951), *Allium longicuspis* Rgl is an endemic species of central Asia and wild ancestor of garlic.

Garlic bulbs are rich sources of carbohydrates, proteins, phenols and antioxidants. Besides this garlic also constitute some other chemicals as additional protection for those insects and animals not deterred by its volatile metabolites sulphur based compound, (+) S-allyl-1 cysteine sulfoxide oralliine. The garlic has been utilized since the very beginnings of Ayurveda, the traditional medical system of India, as an anti-inflammatory, analgesic, antipyretic, sedative, antioxidant, and antiepileptic agent. It acts as curative measure upon cardiac diseases and cancer.

The latest global review of area and production shows that among major vegetables, garlic ranks fifth in area and seventh in production. However in India, natural stand of garlic is found in northern part of country where relatively cool winter and hot and dry summer occurs. Garlic is grown in the northern parts especially in Gujarat, Haryana, Punjab, Madhya Pradesh and Maharashtra, and in a few southern localities, especially the cold hilly tracts of Ooty and Kodai kanal, Tamil Nadu. A total of 1,264 MT garlic is been produced in year 2010-11. Gujarat is the largest producer of garlic in India with production of 275 MT, followed by Madhya Pradesh (228MT production) (Gupta, 2011). Until recently, characterization of garlic germplasm on the basis of morphological traits accomplished worldwide by Bradley *et al.,* 1996, Garcia *et al.,* 2003, Pathee *et al.,* 2006, Buso *et al.,* 2008, Asili *et al.,* 2010, Khar 2012, Singh *et al.,* 2014 etc. In India although crop enjoys worldwide distribution and dynamic importance, its growth habit are restricted to *rabi* season only. Further as major available garlic used as seed material in that particular season, there is glut in market leads to price hike. More above

short day garlic completes its life cycle with in five months so there is alternative to grow the crop twice in year. Hence present experiment planned to check and identify *kharif* season suitable garlic ecotypes among available genetic resources at ICAR-DOGR. In future identified acclimatized genotypes can be purified by clonal selection method for promising trait like high yield, early maturity and bolting free.

Material and method

Present study has been carried out at ICAR-DOGR farm, Rajgurunagar which located at elevation: 645 m   coordinates: 18.8550° N, 73.8875° E, Pune. Total 400 garlic accessions collected from different agro-climatic regions of India (Table 1) has been planted in Latin square design in two replicates during *kharif* 2015 and 2016. In both years out of 400 evaluated accessions, only 66 accessions could able to develop proper bulb however in remaining accessions bulb induction was observed but no longer proper bulb enlargement with lateral bud differentiation. Bulb forming accessions along with its origin/collected state is mentioned in table 1.

Using 66 bulbing accessions, nine quantitative and six qualitative traits were recorded as per DUS guidelines over five representative samples in each replication. Further this replicated data used for analysis of variance using SAS9.3 software in both years. In pooled analysis, as no significant difference was found between years into accession interaction, mean data of both years used for further secondary analysis which includes grouping of genotypes using agro morphological traits, correlation studies among traits, and principal component analysis. Average data was subjected to JMP pro software for clustering analysis and PCA calculations.

Results and Discussion

In case of garlic, a clonal propagated crop knowledge of genetic diversity helps in direct selection of promising genotype and to develop variety. In present experiment after field evaluation of 400 garlic accessions only 66 accessions could able to form bulbing. In rest of accessions only vigorous vegetative growth but no bulb induction observed. This may happened as garlic crop is highly photo and temperature sensitive crop. Initial 50 days after planting crop needs 1) Night temperature between 10 to 15 C 2) Day temperature between 25 to 28 C 3) 70 to 80% humidity in air 4) 11 to 12 hours photoperiod. However in next 30 to 40 days when bulb enlargement and clove differentiation starts crops require lower night temperature and higher day temperature with less humidity compare to previous 50 days.

As *kharif* crop generally planted on 15th June up to 15th July, initial crop growth stages gets simulated environmental conditions alike *rabi* season. But after 50 days of planting, at the critical bulb induction stage humidity get increased and sunshine hours reduced due to uneven rainy days and cloudy climate. The genotypes having inherent capability to cope up with such challenging climatic situations are survived and found promising for *kharif* season. Further there is need to screen the diverse population for bulbing in *kharif* at molecular level to identify related QTL. Molecular level study will help to understand the phenomenon more critically.

In bulbing genotypes, nine morphological traits has been recorded (Table 2) with sufficient variation. Accession number 266,493 and 784 recorded highest yield i.e.5.5 to 5.9 q/ha. In case of bulb size, average bulb weight was 5.81 but promising lines had bulb with more than 10gm bulb weight with ovate size bulb shape. Garlic genotype 499, 553, 50 and 24 are identified as high TSS lines (45.2

to 46.6 degree brix). Highest variance noted in weight of 50 cloves (146.08) as within entire population there is wide divergence in shape and size of bulbs as indicated in polar diameter and equatorial diameter. Among recorded qualitative traits wide variation noted as described in table 3.

Diversity studies

Cluster analysis based on morphological traits is an older breeding tool to get understand genetic resource for selecting genotypes for large scale multiplication and planning crop improvement programme. In cluster analysis, total 66 garlic accessions were primarily grouped into five main clusters based on genetic distance calculated upon fifteen agro morphological traits (Fig. 1). In grouping pattern there was no correlation observed among geographical origin of the accessions and clustering pattern as accessions from same state were clustered into different groups and accessions originated from different states clubbed in one cluster.

Maximum genetic distance (14.44) was recorded between accession 202 and DOGR 499 while minimum genetic distance found in 695 and 14 (0.95). Some similar research could also be referenced. This grouping will helpful to breeder to select trait specific genotypes for further selection and development of variety. Here, cluster number III consists of all high yielding accessions hence contributing traits average bulb weight, polar diameter and equatorial diameter also record highest values (Table 4)

However, cluster I contain accessions with highest weight of 50 cloves. As primarily shown in table, highest TSS recorded by cluster 3, highest TSS lines are present in cluster IV which suggest need to refine grouping on more critical level.

Table.1 Origin/state wise distribution frequency of garlic accessions

Sr. No	Origin state	Total no. of accessions	Code name of accessions
1	Andhra Pradesh	2	367 & 543
2	Arunachal Pradesh	1	698
3	Assam	1	695
4	Bihar	1	730
5	Gujarat	15	1,3,9,14,148,202,279,282,324,347,414,460,489,540 & DOGR-744
6	Haryana	4	51,214,689 & 756
7	Jammu &Kashmir	1	439
8	Madhya Pradesh	6	278,303,621,753,755& 784
9	Maharashtra	7	50,201,266,329,425,493 & 759
10	New Delhi	8	244,257,357,438,443,502,553 & 595
11	Odisha	8	364,432,441, 709, 710, 712, 742 & 742
12	Punjab	1	517
13	Rajasthan	2	534 & 746
14	Sikkim	3	665, 668 & 671
15	Uttarakhand	6	24,187,437,593,DOGR-243 & DOGR-499

Table.2 Trait potential of evaluated genotypes with respect to mean, range, Std. dev. and variance

Sr. No.	Trait	Mean	Range		Std. Dev.	Variance
			Min	Max		
1	Wt. With Leaves(Kg)	0.20	0.10	0.48	0.08	0.01
2	Wt. Without leaves(Kg)	0.17	0.09	0.41	0.07	0.01
3	yield q/ha	3.36	0.81	5.92	0.69	0.48
4	PD(mm)	20.78	17.77	24.28	1.52	2.32
5	ED(mm)	20.96	17.02	30.21	2.98	8.89
6	ABW	5.81	3.48	12.08	1.52	2.33
7	NC/B	7.84	4.40	15.00	2.44	5.96
8	W50C	39.65	16.97	70.35	12.09	146.08
9	TSS	42.23	35.92	46.64	2.41	5.80

(PD- Polar diameter; ED- Equatorial diameter; ABW- Average Bulb Weight; NC/B- Number of Cloves per Bulb;W50C- Weight of 50 Cloves; TSS- Total Soluble Solids))

Table.3 Distribution frequency of qualitative traits among 66 genotypes

Sr. no.	Trait name	Class wise distribution among genotypes
1	Shape of Bulb	Transverse Elliptic- 26; Ovate- 27; Circular-13
2	Colour of Bulb	White- 40; Purple-26
3	Colour of Clove	White-38; Purple-28
4	Bulb Compactness Of Clove	Loose-14; Medium-32; Compact-20
5	Clove -Colour Of Flesh	White- 33; Yellowish-33
6	Bulb Distribution of Clove	Radial-24; Non-radial-42
7	Bulb Shape Of Base	Recessed-16; Flat-33; Rounded-20

Table.4 Cluster-wise mean performance of yield and contributing traits

Traits	Total No of Accessions	MY q/ha	PD (mm)	ED (mm)	ABW	NC/B	W50C	TSS
Cluster I	11	2.23	21.71	23.49	6.86	6.78	52.60	41.92
Cluster II	7	2.21	20.98	22.74	6.71	11.86	24.54	42.22
Cluster III	3	3.83	23.48	29.26	8.91	13.53	32.03	43.16
Cluster IV	33	1.34	21.02	20.12	5.63	7.10	42.28	42.52
Cluster V	14	1.11	18.81	18.29	4.30	7.20	32.46	41.61

(MY- Marketable yield; PD- Polar diameter; ED- Equatorial diameter; ABW- Average Bulb Weight; NC/B- Number of Cloves per Bulb; W50C- Weight of 50 Cloves; TSS- Total Soluble Solids)

Fig.1 Dendrogram showing genetic distance among *kharif* garlic accessions plotted on basis of sixteen traits
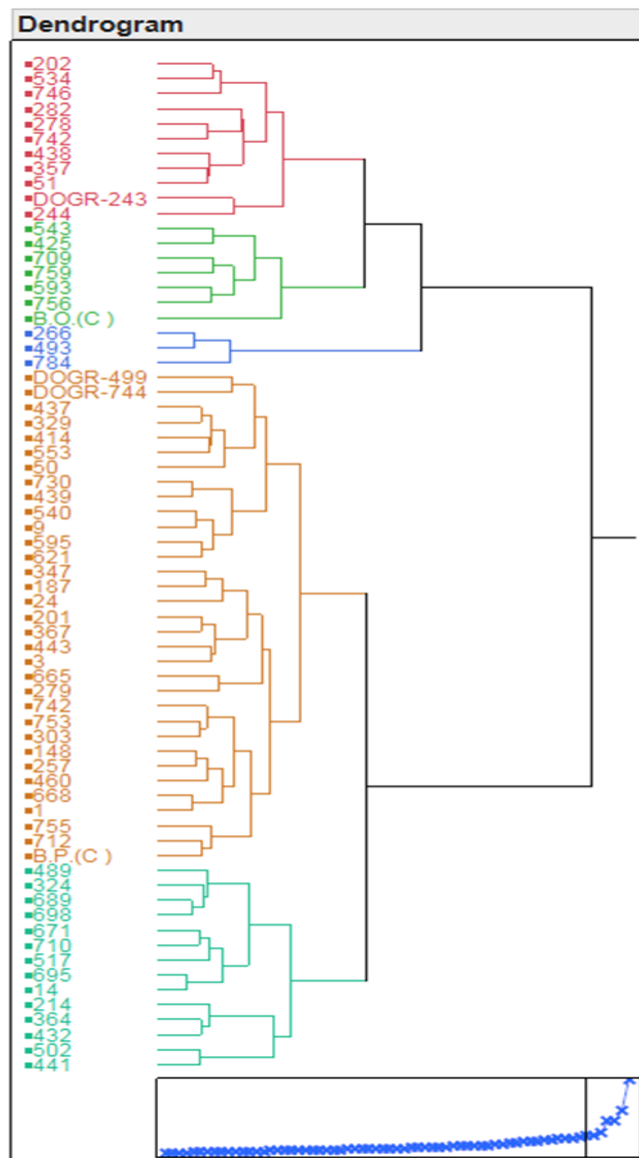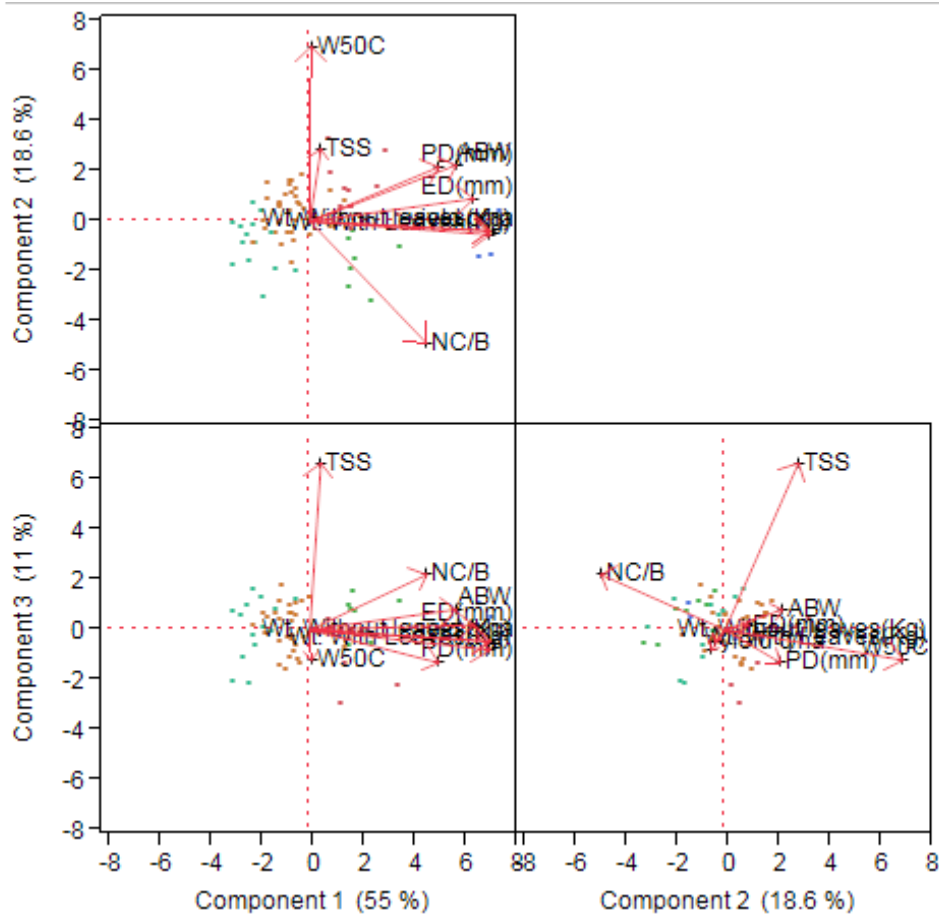
Fig.2 Score plot loading with associated traits for first three components

Principal coordinate analysis based on traits relative with yield clustered 66 genotypes into three groups (Fig. 2). First three components were accounts for 80% of total variation. Major traits such as total yield, average bulb weight, polar diameter and equatorial diameter played important role in contributing 55% variation due to first component with 4.94 eigenvalue however weight of 50 cloves and total soluble solids leads to variation in component second and third with 1.67 and 0.98 eigenvalues respectively. As explained by PCA researcher interested in high yield can concentrate on group first however for getting high TSS lines they have to exploit group three. For both mentioned traits identified genotypes need improvement through clonal selection purification and stability.

Garlic is a clonal propagated crop known for its medicinal and condimental properties. Till date variability in this crop published by countries like China, Brazil, Nepal, Australia, Argentina and USA at agro-morphological, biochemical and Molecular level (Pathee *et al.,* 2006, Garcia *et al.,* 2003, Buso *et al.,* 2008, Bradley *et al.,* 1996, Asili *et al.,* 2010). However in India also different researchers worked on few genotypes along with some varieties of garlic (Singh *et al.,* 2014; Khar. A 2012; Khar *et al.,* 2008). But this paper is a first report of screening India's almost all tropical region representative genotypes for *kharif* season suitability. Although not all genotypes able to form bulb a group of bulbing accessions identified and evaluated for phenotypic traits. Suffice variability in qualitative as well as quantitative traits has

been revealed in two years experiment for yield and other related parameters. In genotypic performance high yielding ecotypes identified, same way through divergence study such genotypes were sorted. Wang *et al.,* (2014) reported that three Chinese accession yielded more than 15t/ha while Panthee *et al.,* (2006) recorded highest yield as 61 t/ha. Besides quantitative traits, qualitative traits too display good amount of variation. CA revealed same results alike clustering where yield and other contributing characters played major role for divergence. Here similar results were observed by Panthee *et al.,* (2006).

Clustering of germplasm based on scored traits is easiest method to identify genetically distant trait specific genotypes. This method has been very popular in cereals like rice, wheat and so on. In garlic also in China 212 accessions sorted into six groups on the basis of morphological traits by Wang *et al.,* (2014), similarly Panthee *et al.,* (2006) revealed three groups from evaluating 179 garlic accessions from Nepal. Menezes Sobrinho *et al.,* (1999) conducted a study to 89 garlic germplasm of Brazil and found 13 clusters. Lallem and *et al.,* (1994) also evaluated 65 garlic accessions and found six clusters on the basis of morphological traits. In present experiment, we have clustered 66 genotypes in five groups where cluster number three defines high yield potential genotypes but for specifying other traits like TSS there is need to sort population more closely. As group III shows highest mean for TSS, genotype DOGR-499 from group IV recorded highest TSS among entire population i.e., 46 degree brix..

Evaluation of garlic genotypes during off season *(kharif)* will definitely lead to open door for alternate cultivation season of crop. This experiment able to screen and identify the promising accessions for yield and related traits. Further improvement in traits will give a garlic variety for *kharif* season. This ultimately enhances production of garlic in India. Rather helpful to manage the price hike when no garlic was chiefly available for consumption to normal community.

Acknowledgement

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How to cite this article:

Ashwini P. Benke, Somnath Dukare, Vijay Mahajan and Major Singh. 2018. Genetic Divergence Studies for Bulbing and Related Traits in Garlic Germplasm during *Kharif* Season. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 2920-2927. doi: https://doi.org/10.20546/ijcmas.2018.701.349