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Variability, divergence and SAHN grouping studies in extra-large seeded Kabuli chickpea (*Cicer arietinum* L.) genotypes

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Abstract

There exists limited information on diverse genotypes available for breeding programme in Extra-Large Seeded Kabuli (ELSK) types. Of recent there had been a great demand in India towards the import of ELSK chickpea due to increased consumer demand. Department of Agriculture and Co-operation, government of India funded a programme on Integrated Scheme on Oilseeds, Pulses, Oil Palm and Maize (ISOPOM) project in which ELSK chickpea was one of the component and had an objective of evaluating divergent ELSK chickpea genotypes. Divergence studies in eighteen Extra-Large Seeded Kabuli (ELSK) genotypes done through Sequential agglomerative hierarchical nested cluster analysis (SAHN) of NTSYS-PC software (version 2.21b) indicated presence of considerable variability among the lines studied. Seed yield formed the primary divergent character for cluster formation and sub-grouping within a cluster followed the seed weight pattern. The clustering pattern grouped the genotypes into two major clusters and the ELSK genotypes PG4303 and KAK 2 of cluster 1 were most divergent to that of AP2 of cluster 2.

Keywords: Variability, divergence, SAHN, Kabuli chickpea, *Cicer arietinum* L., genotypes

Introduction

Chickpea (*Cicer arietinum* L.; Family: Fabaceae) a self-pollinated, diploid ($2n=16$), cool season pulse crop with a genome size of ~738-Mb with an estimated 28,269 genes (Varshney *et al.*, 2013) and is widely grown in more than 50 countries representing all the continents (Upadhyay *et al.*, 2011). Chickpea is grown mostly in South Asia and Sub-Saharan Africa, which accounts for more than 75% of the world chickpea area. Global chickpea production has increased from 7.68 million tonnes (1961) to 13.73 million tonnes (2014) (FAOSTAT, 2016). India ranks first in terms of cultivated area and production. However, there is a slight increase in the chickpea cultivation area in India from 9.27 million hectares (1961) to 9.92 million hectares (2014), but production increased significantly from 6.25 million tonnes (1961) to 9.88 million tonnes (2014) due to significant increase in the productivity from 0.67 t/ha (1961) to 0.99 t/ha (2014). Other major chickpea producing countries are Australia (629,400 tonnes), Myanmar (562,163 tonnes), Ethiopia (458,682 tonnes), Turkey (450,000 tonnes), and Pakistan (399,030 tonnes) (2014) (FAOSTAT, 2016). However, India still continues to import chickpea, particularly the kabuli bold type. There is an urgent need to look into this important aspect and breed for varieties with higher market demand. The food and feed demand for Indian population are not only expanding but also changing and pulses are forming a greater part of the diet along with vegetables to the pre-dominantly vegetarian population in India. India also imports around 300,000-400,000 ton chickpea per year. India accounts for over 30% of all imports, almost all desi. Pakistan, Spain, and Bangladesh are the other three major importers. India and surrounding countries import mainly the desi type, while countries in North and South America, Europe, the Middle East and Africa import mainly the kabuli type. The price difference between desi and kabuli is partly related to the end user market. Kabuli chickpeas tend to be used in relatively more affluent countries. Desi's are primarily consumed on the Indian sub-continent where purchasing power isn't as great. Desi prices generally track edible yellow pea prices but at a considerable premium. However with increasing income and purchasing power, demand for extra-large seeded kabuli types is steadily increasing. India being the largest importer, it is driving the demand in international market for extra-large

(seed size of >50g for 100 seeds) whose prices fetch as high as three times the desi prices. A large demand and scarcity of production for extra-large seeded kabuli types exists, forcing the traders to import extra-large seeded kabuli (ELSK) types into the country and the farmers to grow unsuitable ELSK varieties. This can be overcome by development and deployment of appropriate production technologies as well as new improved varieties. Traditionally most of the breeding projects aimed at *desi* types which still rule 80 per cent of market demand. Though many varieties exist before the farmer in selecting the varieties suitable for his needs, he has a limited basket to select upon when he wants to grow ELS Kabuli varieties.

An insight into diversity among available ELSK lines would provide valuable guidance to the breeders and germplasm people for looking into the diversity available in elite ELSK lines/ varieties and make elite x elite crosses to achieve greater yield gains. Further such a genetic diversity would also help in increasing the genetic base of the elite breeding material. It has been well established that genetically diverse elite lines are likely to yield better desirable gene recombinants and also do not carry much genetic load associated with pre-breeding material. Geographical diversity was often used as an index of genetic divergence. Availability of multivariate analysis has overcome this handicap and these techniques were employed by many workers to discern the genetic distance (Bharadwaj *et al.* 2007) [2]. Comparing different multivariate analyses techniques, Bharadwaj *et al.* 2001 [1] reported the advantage of principal component technique. Sequential, agglomerative, hierarchical, and nested (SAHN) clustering methods using as defined by Sneath and Sokal (1973) [6] better resolves these distances as it includes such commonly used clustering methods as UPGMA and single-link using the Euclidean distances. This program when run in NTSYS-PC software (version 2.21b) it can find alternative trees when there are ties in the input matrix. Keeping the above points in mind our study was mainly involved in studying the diversity among the extra-large seeded kabuli (ELSK) elite lines and varieties that have been tested in chickpea under the Integrated Scheme on Oilseeds, Pulses, Oilpalm and Maize (ISOPOM) project of Department of Agriculture and Co-operation, Government of India.

Materials and Methods

The plant material consisted of eighteen ELSK accessions that were obtained under the ISOPOM project from Crop Improvement Section, Indian Institute of Pulses Research, Kanpur and being maintained at chickpea breeding unit, ICAR-IARI. This material was basically used as the elite material at IARI for developing extra-large seeded kabuli convergence cross programme. Hence a need was necessitated to test these lines for the variability and diversity present. The material mostly consisted of elite lines, released varieties and farmers accessions (Table 1). The material was planted in randomised block design with three rows per replication in three replications and the mean data of randomly selected and tagged five plants from the central row was used for analysis. Tree construction was done through Sequential agglomerative hierarchical nested cluster analysis (SAHN) of NTSYS-PC software (version 2.21b). The mean data for the traits days to 50 % flowering, days to maturity, plant height, 100 seed weight and yield per plant was used in the analysis. The Euclidean coefficients of the means were used in calculating the unweighed paired group mean averages for the construction of the tree.

Results and Discussion

A survey of genetic variability is essentially a first step in crop improvement and plant breeding is an exercise in the management of variability. A collection of lines or breeding materials by itself is of little value unless it is assessed or evaluated for characters of interest. Since the interest in ELSK chickpea in India is a recent one and the basic information needed for selection of lines for the improvement of ELSK chickpea is lacking, hence evaluation of available lines forms the first step of the improvement programme. In the present study 18 genotypes were subjected to diversity analysis. The *per se* performance of ELSK chickpea genotypes revealed that there was a substantial variability among genotypes for all the characters. A high degree of diversity polymorphism was exhibited by all the characters that have been analysed using the Sequential agglomerative hierarchical nested cluster analysis (SAHN) of NTSYS-PC software (version 2.21b) indicating considerable variability in the material under study. Even the analysis of variance (Table 2) indicates the presence of considerable amount of exploitable variability. The SAHN coefficients ranged from dendrogram constructed using the UPGMA method showed the genetic similarity between cultivars ranged till a range of 601 (figure 1) indicating the grouping so obtained would be stable even in addition of newer characters and there is less chance of a change in this grouping pattern.

This study revealed that all the 18 varieties grouped into two major clusters. A critical examination of these two clusters clearly indicate that the grouping was primarily based on seed type viz., desi or kabuli and within a group the molecular diversity by descent appears to play a major role as the varieties with same ancestors tended to go together.

The comparison of genetic relationship pattern obtained by STMS matrix data with that of morphological and breeding station entry information available for 18 lines showed a clear cut pattern of clustering that emerged based on yield and seed size which contributed maximum ranks to the diversity grouping. The eighteen genotypes formed two distinct groups with nine genotypes in each subgroup. Association of clustering appears to yield and seed weight in the SAHN grouping, which is supported by high similarity coefficient values (Fig 1) among the genotypes for these two traits within a cluster. Rakesh Singh *et al.* (2008) [7] reported similar clustering pattern based on seed type and flower colour while studying diversity using molecular markers while Bharadwaj *et al.* (2010) [3-4] reported ancestry and seed size as one of the main characters in grouping chickpea genotypes. Breeding for extra-large seed Kabuli requires identification of proper diverse germplasm to generate better breeding material for utilization and selection programmes. However such studies in ELSK lines available in India are meager. Non-availability of such information generally makes the breeder use material from divergent sources for crosses with a hypothetical inference of them being divergent. This study identifies the divergent material that has been collected and evaluated conclusively for three seasons in the ISOPOM project. The genotypes AP5, AP3, IPCK2004-1, AP4, PG 95333, VIRAT, PG 4301, KAK 2 and PG 4303 grouped into a sub cluster with greater coefficient of similarity between KAK2 and PG 4303. SIMILARLY IPCK 02, CSJK 21, IPCK 01, JKG 2004-334, Dollar, PG 516, PG 515, PG 517 and AP2 grouped into a second sub cluster. The sub clustering pattern and grouping pattern followed agglomeration at the yield level followed by seed weight. These two have contributed maximum to the genetic diversity among the characters. The genotype AP2

which had the lowest yield level of 1129 kg.ha⁻¹ was the farthest away from all other genotypes and farthest from KAK2 which had a yield level of 2242 kg.ha⁻¹. In cluster 1 the range for yield was from 1758 kg.ha⁻¹ (AP5) to 2242 (KAK2) kg.ha⁻¹ while in cluster 2 the yield levels ranged from 1129 (AP2) to 1708 (IPCK02) kg.ha⁻¹. All the genotypes in cluster 1 had yield levels above 1708 kg.ha⁻¹ while those in cluster 2 had yield levels below this. It has been clearly observed that grouping did not follow geographical location or the breeding station from where the lines have been obtained clearly indicating that geographical diversity is not an indicator of genetic diversity. Similar findings were reported by Bharadwaj *et al.* 2001^[1], Tara Satyavathi *et al.* 2005 and Bharadwaj *et al.* 2010^[3-4], 2011. Seed weight which ranked second in its contribution to diversity was one of the characters that contributed to sub-grouping within a cluster. The higher seed weight high yielding genotypes of cluster 1 were nearer in branching to the higher seed weight, low yielding genotypes of cluster 1 and were at a closer distance from node. The seed weight ranged from 35 (KAK 2) to 64

(AP5) for 100 seed weight in cluster 1 to that of 41 (JKG2004-335) to 55 (AP2) in cluster 2. Without clustering done, a breeder would have selected KAK2 with AP5 for crossing. However, the clustering pattern indicates that greater gains are bound to be obtained from crossing PG4303 or KAK 2 of cluster 1 with AP2 of cluster 2 and there is likely hood of getting transgressive segregants from such a cross. The success of any breeding program depends upon the availability of adequate genetic diversity. The major factor responsible for limited success in increasing the chickpea yield particularly in ELSK trials has been the narrow genetic base of the material available. Plant breeders are always interested in assessing the genetic divergence in the germplasm or advanced breeding lines available with them so as to utilize them in directed breeding programmes. The present investigation supplements this information and identifies divergent sources of extra-large seeded kabuli chickpea genotypes adapted to Indian conditions for their utilization in breeding programme.

Table 1: Extra-large seeded chickpea lines used in diversity analysis with the means of character traits

S. No	Line	Source	Days to 50% FI	Days to Maturity	Plant height	100 seed wt.	Yield kg/ha
1	AP 4	RARS, Nandyal, Andhra Pradesh, India	64.67	120.67	65.00	49.47	1818.67
2	AP2	RARS, Nandyal, Andhra Pradesh, India	52.00	120.00	62.53	55.33	1129.00
3	AP3	RARS, Nandyal, Andhra Pradesh, India	51.67	120.00	59.20	49.77	1800.33
4	AP5	RARS, Nandyal, Andhra Pradesh, India	64.33	119.67	64.87	52.40	1758.67
5	CSJK 21	Durgapura, Rajasthan, India	65.00	119.33	63.33	52.43	1599.67
6	Dollar	IARI, New Delhi	56.33	120.33	65.43	49.40	1452.33
7	IPCK01	IIPR, Kanpur, India	52.00	121.00	63.43	48.97	1513.00
8	IPCK02	IIPR, Kanpur, India	51.67	122.33	66.67	50.30	1708.00
9	IPCK2004-1	IIPR, Kanpur, India	61.67	120.67	59.67	45.33	1800.67
10	JKG 2004-334	JNKVV, Jablapur, India	57.33	120.00	50.00	41.13	1452.67
11	KAK 2	ICRISAT, Hyderabad, India	58.67	120.00	48.33	35.77	2242.67
12	PG 4303	PDKVV, Akola, India	60.00	122.00	60.00	40.13	2342.67
13	PG4301	PDKVV, Akola, India	63.33	121.00	59.00	40.50	1964.67
14	PG515	PDKVV, Akola, India	60.67	119.00	55.20	51.00	1390.67
15	PG516	PDKVV, Akola, India	64.33	121.00	66.77	50.57	1402.67
16	PG517	PDKVV, Akola, India	60.00	119.00	57.20	52.43	1356.00
17	PG95333	PDKVV, Akola, India	60.67	119.00	72.33	39.03	1932.00
18	VIRAT	PDKVV, Akola, India	63.33	119.00	50.00	32.53	1964.33

Table 2: Anova for 5 quantitative characters in eighteen Extra Large Seeded Kabuli Oines of Chickpea

Source	Mean sum of squares					
	DF	Days to 50% FI	Days to Maturity	Plant height	100 seed wt.	Yield kg/ha
Replication	2	1.87	2.78	33.45	0.01	4.015
Treatment	17	118.62**	8.12**	451.24**	1.52**	55.23**
Error	34	1.01	2.31	15.71	0.006	3.184

** Significant at 1% level of significance.

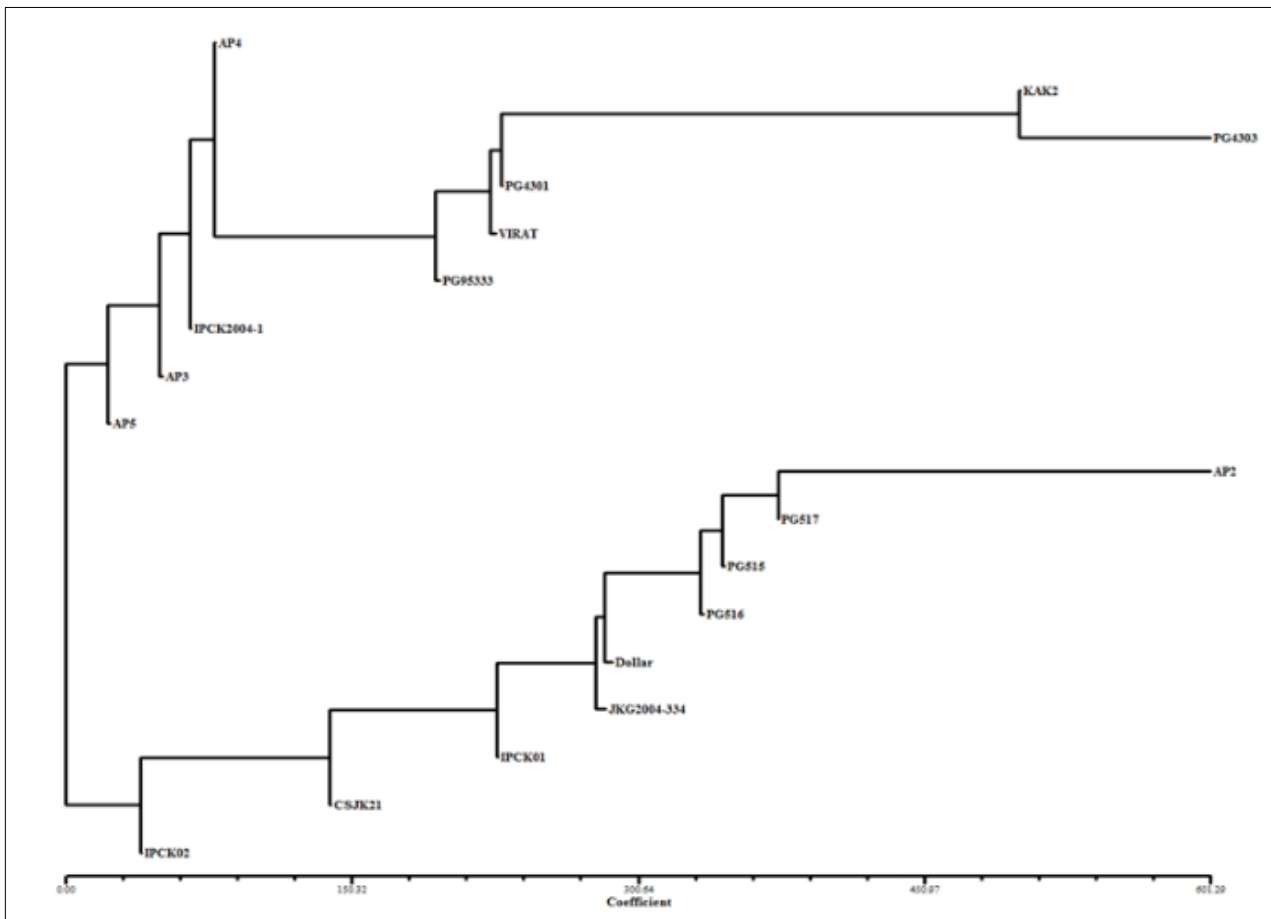


Fig 1: Clustering of eighteen Extra Large Seeded Kabuli Genotypes of chickpea using SAHN clustering method

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