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Research Article

DIVERSE PEA SEED SHAPED RIL'S DERIVED FROM RECOMBINATION BETWEEN ANGULAR AND OWL'S HEAD PARENTS IN CHICKPEA (*Cicer arietinum* L.)

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Abstract- Two hundred fifty two recombinant inbred lines (RILs) generated from recombination between microsperma and macrosperma and their parents were evaluated at RAK College of Agriculture, Sehore during *rabi* 2011-12 and 2012-13. Out of 252 RILs, 92 (36.5%) RILs were identified as pea-shaped seed. These pea-shaped RILs showed wide variations in qualitative traits such as plant growth habit (semi-erect 10RILs; semi-spreading 32; spreading 46 & prostrate 4), flower colour (pink 65; white 25; blue 2), seed surface (smooth 75; rough 17), seed colour (brown 39; yellow 30; orange 5; creamy-white 18). These 92pea-shaped RILs were grouped into 15 clusters {clusters I (6 RILs), II (2), III (6),IV (6), V (17), VI (1), VII (9), VIII (10), IX (6), X (5), XI (8), XII (7), XIII (1), XV (5), XVI (3)}confirming their genetic diversity and distinctness in quantitative traits. The spectrum of variations in pea-shaped RILs was beyond expectation was due to recombination and segregation due to high parental diversity. These RILs will be used for development of high yielding and marketable pea-shaped chickpea varieties.

Keywords- Chickpea, pea-shaped RILs, genetic variability and diversity.

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Introduction

Chickpea is the third most important food legumes crop in the globe in area (11.55 million hectares) and production (10.46 million tons) [1]. It is also a rich source of protein, enrich the soil through biological nitrogen fixation and can sustain under rainfed situation. The genus *Cicer* comprises 43 species which shares 42 wild (34 perennials and 8 annuals) and 1 cultivated species *Cicer arietinum* L. [2, 3]. It is normally self pollinated but 1% cross pollination has also been reported by [4]. In grain legumes like chickpea, narrow genetic variability is limiting factor in achieving high productivity [5]. When the parents used in a cross are genetically similar, it is quite likely that the different lines derived reveal low diversity. On the contrary, when diverse parents are used in obtaining a segregating population, the derived lines reveal greater diversity [6]. Keep this view in mind, 252 RILs, which were derived from recombination between diverse parents; macrosperma (ICC 283) and macrosperma (ICC 8261) were evaluated to identified desirable pea seed shaped RILs. Diversity in pea shaped RILs were measured and lines identified have been discussed in this investigation.

Materials and Methods

The experimental material consisted of 252 recombinant inbred lines (RILs) derived from cross between microsperma (ICC 283) and macrosperma (ICC 8261) parents were grown in randomized block design with two replications. Each entry was sown in 4 m long single row with 30 cm row-to-row and 10 cm plant-to-plant spacing at R.A.K. College of Agriculture, Sehore (M.P.) during *rabi* 2011-12 and 2012-13.The fertilizer dose 20:50:0:20 NPKS kg/ha was applied as basal dose. International Chickpea Descriptor [7] was used as guideline for recording qualitative and quantitative traits. Observations on qualitative traits were recorded on the basis of individual lines performance and quantitative traits recorded on five randomly selected plants from each replication and each condition. D^2 statistic [8] and Tocher's method [9] for cluster analysis were adopted for standard statistical analysis and their interpretation.

Results and Discussion

An attempt was made to know the genetic variability and diversity among the RILs based on qualitative and quantitative traits. It is important to point out that RILs were divided into three groups on the basis of seed shape *viz.,* angular shape, owl's head and pea-shaped. Out of 252 RILs, 92 RILs were identified as pea-shaped with wide spectrum of variation for other qualitative traits *viz.,* stem pigmentation, leaflet size, leaflet colour, number of leaflets per leaf, flower colour, plant growth habit, seed colour and seed surface. Further seven quantitative traits *viz.,* days to 50% flowering, days to maturity, plant height, biological yield, seed yield, harvest index, hundred seed weight also recorded for diversity amongst the RILs.

Seed shape is one of the most important traits used for identification and categorization of chickpea across the globe into *desi* and *kabuli* group. RILs were identified as angular shape (36.9%), owl's head shape (26.6%) and pea-shape (36.5%) [Table-1], [Fig-1] and [Plate--1]. Variations in seed shape and colour obtained in different RILs can be used for future recombination breeding programme and new dimension for the enhancement of pea seed shape chickpea varieties. The literature available in relation to the above observations includes that of [10]. According to him, the inheritance study of seed shape in chickpea indicated that pea seed shape is dominant over both *desi* and *kabuli*, and *desi* is dominant over *kabuli* shape. In this investigation, it was observed that *desi* parent had angular shape seed and *kabuli* parent with owl's head shape seed and frequency of *desi* type RILs is higher as compared to *kabuli.* These observations

are similar to those, reported by [10].

Table-1 *Classification of 252 RILs on the basis of seed shape*

S. No.	Seed shape	RILs		Parents
		Frequency	Percentage	
1	Angular	93	36.9	ICC 283
2	Owl's head	67	26.6	ICC 8261
3	Pea-shaped	92	36.5	

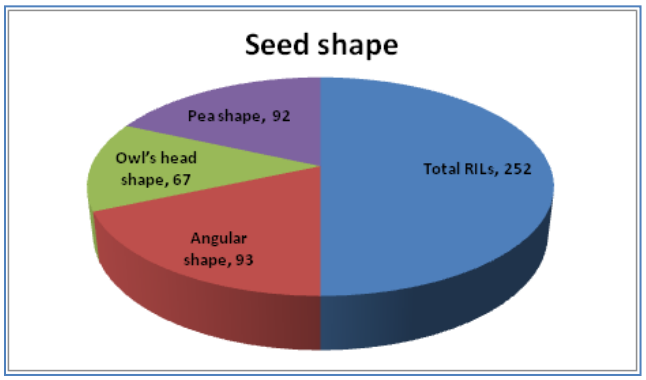

Fig-1 Showing relative frequencies of seed shape in 252 RILs

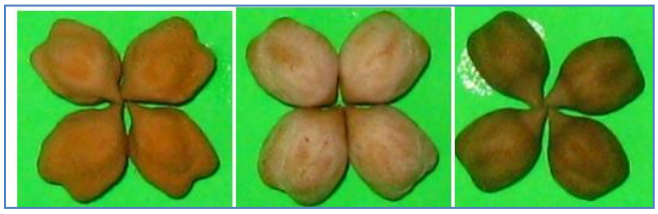

Plate-1 Showing angular, owl's head and pea seed shape in RILs

Stem pigmentation is used as a marker trait for identification of varieties. Maximum number of 46 pea shape RILs showed no stem pigmentation and its frequency was 50%, 19 RILs (20.7%) showed low stem pigmentation and remaining 27 RILs (29.3%) showed medium pigmentation [Table-2] and [Plate-2]. Pea shaped RILs categorized into three types of leaflet size *viz.,* small, medium, large. Highest frequency of medium leaflet size was recorded in 53.3% pea-shaped RILs followed by small leaflet size [Table-2] and [Plate-3]. Small leaflet size is considered as one of the desirable traits as compared to broad and medium leaflets for drought tolerant capacity as they have low water transpiration rate. RILs with small leaflets can be utilized as major contributor for the development of moisture stress tolerance variety in chickpea. [11-13] also reported variations in leaflet size in chickpea germplasm as has also been observed in the present investigation. Colour of leaflets play an important role in identification of *desi* and *kabuli* varieties in chickpea. Leaflet colour was grouped into two categories *i.e.* dark green and light green. Out of 92 pea shape RILs, 67 RILs (72.8%) were recorded with dark green and 25 RILs (27.2%) with light green category [Table-2] and [Plate-4]. In this investigation nno new recombinants generated for leaflet colour but presence of light leaflet colour in pea-shaped RILs will help in breeding programme because light leaflet colour is not common in *desi* group. Chickpea is having compound leaf type and number of leaflet varies in different varieties. It was classified into two categories *i.e.* 11-13 and more than 13 leaflets per leaf [Table-2] and [Plate-5]. Flower colour plays an important role in identification of *desi* and *kabuli* type chickpea in majority of cases. It was classified into three categories *viz.,* pink, white and blue [Table-2] and [Plate-6]. RILs with blue flower are identified as new lines which can be utilized for identification of varieties and useful in further breeding programme. Interaction of two recessive factors involved in light blue corolla in chickpea found by [14]. Flower colour variation as a fixed trait used for germplasm characterization reported by [15,12]. Monogenic inheritance pattern of pink vs. white colour flower in RILs (ICCV2 x JG 62) of chickpea was reported by [16]. Among 92 pea shape RILs;46 RILs (50%) recorded as spreading type followed by 32 RILs (34.8%) with semi-spreading, 10 RILs (10.9%) with semi-erect and 04 RILs (4.3%) with prostrate [Table-2] and Plate-7]. Fourteen RILs were recorded as new recombinants type with semi-erect and prostrate plant growth habit. Prostrate plant growth habit is a prominent trait in wild relatives of chickpea and semi-erect growth habit is one of the most suitable traits for more number of plants per unit area and suited to mechanical harvesting. In cognizance of the above, other studies may be mentioned here *viz.*, [14] reported mono-factorial recessive gene inherited prostrate growth habit in chickpea. Seed colouris considered as most appropriate phenotypic trait for identification of *desi*, *gulabi* and *kabuli* varieties. Seed colour was recorded when seeds became physically mature. Out of 92 pea-shaped RILs, nearly 39 RILs (42.4%) showed brown seed colour, 30 RILs (32.6%) with yellow colour, 5 RILs (5.4%) with orange colour and 18 RILs (19.6%) with cream colour were identified [Table-2] and [Plate-8]. Seed colour plays as major factor in market price of chickpea; hence pea-shape RILs generated in this investigation will be utilized as a major trait for commercial exploitation. Coloured seed coat was dominant over salman white seed coat and reported this coating was probably controlled by three pairs of genes found by [16]. Seventy five RILs (81.5%) having smooth surface and 17 RILs (18.5%) with rough surface of seed [Table-2] and [Plate-9]. Rough surface seed is a desirable trait and used for the development of tolerant cultivar against stored grain pest. Hence pea shape RILs with rough surface seed will also bring a major breakthrough in development of chickpea cultivars tolerant against store grain pest. Pea seed shaped RILs generated from angular shape (microsperma; ICC 283) and owl's head shape (macrosperma; ICC 8261) indicating the recombination and reshuffling of genes responsible for seed shape in this investigation.

Table-2 *Parental type and recombinant type plant traits among pea shaped RILs*

S. no.	Qualitative traits of Pea shape RILs	Similarity with Parent I (angular shape)		Similarity with Parent II (owl's head shape)		Dissimilarity from both parents	
		Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
1	Stem pigmentation	19	20.7%	46	50%	27	29.3%
		Low pigmentation		No pigmentation		Medium pigmentation	
2	Leaflet colour	67	72.8%	25	27.2%	00	00
		Dark green		Light green			
3	Leaflet size	49	53.3%	8	8.7%	35	38%
		Medium		Large		Small	
4	No. of leaflets per leaf	85 (92.4%)				7	7.6%
		15 leaflets per leaf				11-13 leaflets per leaf	
5	Flower colour	65	70.7%	25	27.2%	2	2.1%
		Pink		White		Blue	
6	Plant growth habit	32	34.8%	46	50%	10 + 4	15.2%
		Semi-spreading		Spreading		Semi-erect & Prostrate	
7	Seed colour	39	42.4%	18	19.6%	35	38%
		Brown		Creamy-white		Yellow, orange	
8	Seed surface	75 (81.5%)				17	18.5%
		Smooth				Rough	

Plate-2 Showing pigmented and non pigmented stem in RILs

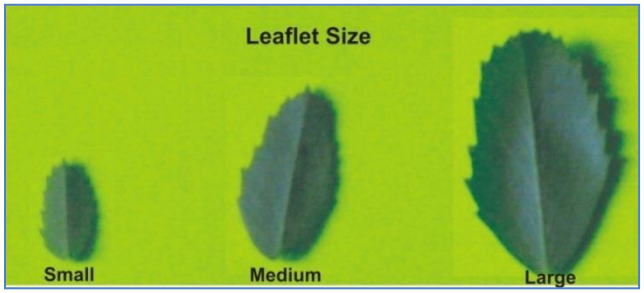

Plate-3 Showing small, medium and large size of leaflet in RILs

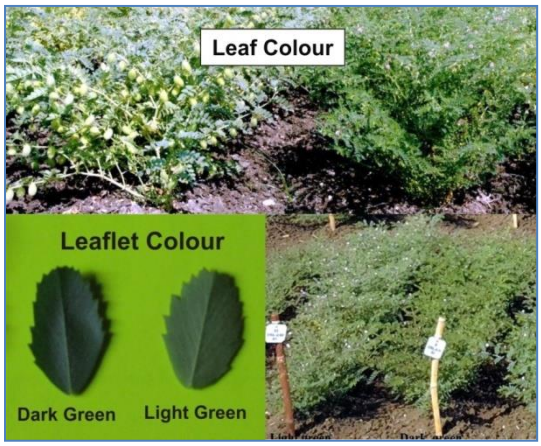

Plate-4 Showing dark and light green colour of leaflets in RILs

Plate-5 Showing number of leaflets per leaf in RILs

The analysis of variance showed significant difference among the RILs and their parents for quantitative traits [Table-4]. Hence, assessment of genetic variability of yield and its contributing traits in RILs and parents was an important target in the present investigation. Variations in RIL population of chickpea are reported by [17-19] also reported similar findings for yield traits in chickpea.

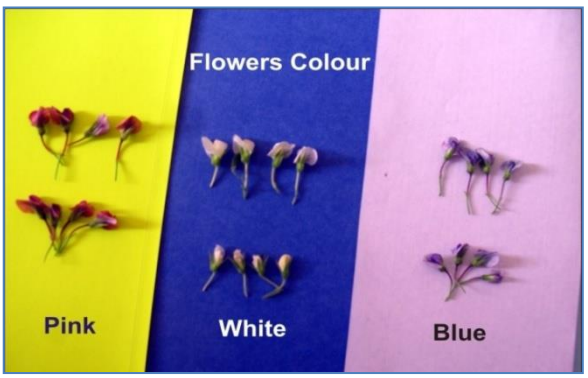

Plate-6 Showing colour of flowers in RILs

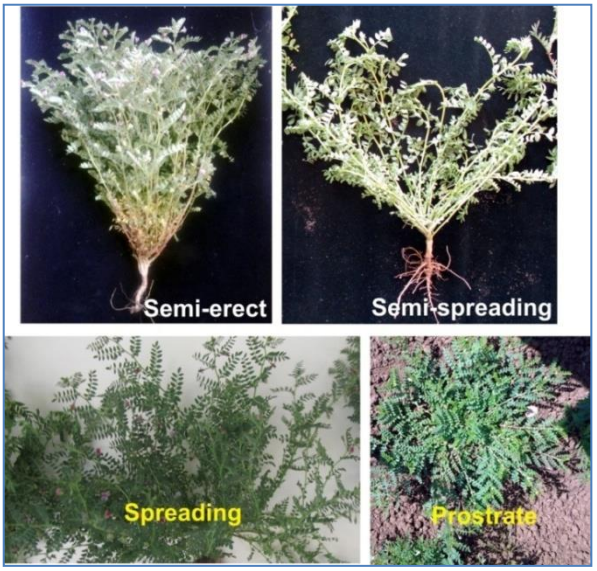

Plate-7 Showing semi-erect, semi-spreading, spreading and prostrate plant growth habit in RILs

Plate-8 Showing different seed colour in pea shape RILs

Plate-9 Showing smooth (Left) and rough (Right) seed surface in pea shape RILs

Table-3 *Spectrum of variations in qualitative traits in 92 pea-shaped RILs*

Plant traits	Type of plant traits	Pea shape RILs	
		Frequency	Percentage
Stem pigmentation	Absent	46	50%
	Low	19	20.7%
	Medium	27	29.3%
Leaflet colour	Dark green	67	72.8%
	Light green	25	27.2%
Leaflet size	Small	35	38%
	Medium	49	53.3%
	Large	8	8.7%
No. of leaflets per leaf	11-13 leaflets per leaf	7	7.6%
	More than 13 leaflets per leaf	85	92.4%
Flower colour	Pink	65	70.7%
	White	25	27.2%
	Blue	2	2.1%
Plant growth habit	Semi-erect	10	10.9%
	Semi-spreading	32	34.8%
	Spreading	46	50%
	Prostrate	4	4.3%
Seed colour	Brown	39	42.4%
	Yellow	30	32.6%
	Orange	5	5.4%
	Creamy-white	18	19.6%
Seed surface	Smooth	75	81.5%
	Rough	17	18.5%

The frequencies of pea-shape RILs in a cluster based on similarities and dissimilarities varied from 1 to 17 RILs in various clusters. The total 92 pea-shape RILs and parents were grouped into fifteen clusters. The relative frequencies of pea-shape RILs in different clusters were recorded as 6, 2, 6, 6, 17, 1, 9, 10, 6 + P I, 5, 8, 7, 1, 00 + P II, 5 and 3 in clusters no. I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV and XVI., respectively [Table-5].The maximum number of 17 RILs were present in cluster V, followed by clusters VIII (10 RILs), VI (9 RILs), XI (8 RILs), XII (7 RILs), I, III, IV & IX (6 RILs each), X, XV (5 RILs each), XVI (3 RILs), II (2 RILs) and only1 RIL was present in cluster VI& XIII each. Kumar *et al*., (2003) grouped 28 genotypes of chickpea into three distinct non-overlapping clusters (I-24 entries, II-3 and III-1). Hundred seed weight followed by days to 50% flowering, plant height, seed yield, days to maturity, harvest index and biological yield were major traits contributing towards generation of diversity in RILs. The pattern of distribution of RILs revealed that considerable genetic diversity was generated due to reorganization of linkage groups and reshuffling of major and minor genes from microsperma parent to macrosperma and vice versa.

Table-4 *Analysis of variance for seed yield and yield contributing traits in 252 RILs & parents*

Source of Variation	d.f	Days to 50 % Flowering	Days to Maturity	Plant height (cm)	Biological yield / plant (g)	Seed yield/ plant (g)	Harvest index (%)	Hundred seed weight(g)
				Mean sum of Square				
Replication	1	5.74	1.43	20.10	8.88	0.15	2.34	0.42
Genotype (RILs + Parents)	253	101.6**	32.2**	45.1**	63.5**	14.0**	123.6**	47.3**
Error	253	6.3	2.7	15.7	10.7	1.5	9.6	2.1

** Significant at 1% level of significance

*Total RILs including angular, pea and owl's head seed shaped generated from cross between angular and owl's head shaped parents.

Table-5 *Relative frequencies of total RILs and pea shaped RILs in different clusters grouped using D² and Tocher Method*

Cluster No.	Frequency of total RILs*	Frequency of pea-shape RILs	Name of Pea-shape RILs
I	20	6	RILs144, 95, 188, 81, 209 and 159
II	12	2	RILs 208and 28
III	18	6	RILs142, 181, 104, 195, 119 and 166
IV	15	6	RILs 8, 23, 92, 63, 125 and 137
V	35	17	RILs 138, 185, 176, 242, 39, 134, 24, 154, 191, 22, 241, 244, 168, 54, 77, 109 and 245
VI	7	1	RIL 116
VII	20	9	RILs 19, 114, 87, 136, 103, 140, 222, 223 and 238
VIII	17	10	RILs 31, 94, 139, 203, 199, 112, 206, 57, 221 and 202
IX	19	6	RILs 86, 79, 225, 200, 120, 193 and **ICC 283 (Parent I)**
X	10	5	RILs37, 21, 224, 178 and 243
XI	21	8	RILs 40, 106, 146, 41, 147, 211, 99 and 91
XII	18	7	RILs 6, 231, 14,160,212, 172, 229 and 228
XIII	6	1	RIL 15
XIV	7	00	**ICC 8261 (Parent II)**
XV	19	5	RILs 70, 205, 121, 50 and 108
XVI	8	3	RILs 67, 110 and 150

*Including angular, owl's head and pea seed shaped RILs

Conclusions

RILs derived from the crossing between two diverse *Desi* (microsperma) and *Kabuli* (macrosperma) parents have generated remarkable new recombinants proven by appearance of pea-shaped RILs and again significant variability and diversity in qualitative and quantitative traits in round or pea seed shape bearing lines. These new pea-shaped RILs will provide the diverse genetic material for the improvement of pea seed shaped varieties. The pea shaped RILs generated with variation in qualitative and quantitative traits will bring a major breakthrough in

long awaited pea shaped chickpea variety development programme in India.

Abbreviations:
RILs: Recombinant inbred lines

Author Contributions: All author equally contributed.

Conflict of Interest: There is no any conflict of Interest of corresponding authors and all authors for Publication of Article. All authors agree for publication of their names in Article.

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Conflict of Interest: None declared

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