LR-5025
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RESEARCH ARTICLE Legume Research- An International Journal

A Pragmatic Study on Seed Shape Classification and its Association among Seed Quality Attributes in Chickpea (*Cicer arietinum* L.)

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10.18805/LR-5025

ABSTRACT

Background: Seed shape in chickpea, a less known but not a trivial trait to study, as the possibility to include it in applied breeding aspect is yet to be deciphered. The present study mainly focuses on the study of seed shape classification and its interrelationship with seed quality parameters.

Methods: The present study was conducted at the N.E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand. Seed shapes in chickpea were classified into distinct classes like angular, globular and owl headed types, using set of genotypes that constituted all the three seed shapes and corresponding hybrids obtained by crossing them.

Result: The Codominance of radicle length and dominance nature of shape of chalazal end was observed in a definite pattern. Angular types were dominant over owl-head and globular types, whereas the globular type was dominant over owl-head type. Angular and globular types had low and high protein content respectively. Globular types would be a key to merge the bi-directionally acting seed traits like protein content and seed weight. From our observations seed shape can be a potential criteria to select for seed protein content. We made clear cut shape classification based on seed components, which could helps in seed shaped based selection in large populations.

Key words: Chickpea, Protein content, Seed shape, Seed size, Seed weight.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), an annual legume crop, belongs to the family Fabaceae and subfamily Faboideae (Gautam *et al*., 2021). Global area under chickpea is 14.84 million ha with 15.08 million tons production (FAOSTAT, 2020). In India, the chickpea production is about 11.08 million tons from an area of 10.94 m ha (FAOSTAT, 2020). Seed shape is an important seed attribute which directly and indirectly incur certain effect on other seed related traits. Chickpea descriptors by IBPGR (1993) explained the seed shape into three categories as angular, Owl-head and round shaped. It is noteworthy, that most of the commercial varieties are owl-headed with low to average protein content. Wood and Keir (2008) formulated a method to classify seed shape based on sphericity of the seed. Hosaain *et al*. (2010) devised the roundedness index to classify the seed shape in chickpea. To include seed shape as a selection component in breeding programmes, clear shape classification system is required. Wood and Keir, (2008) and Knights *et al.* (2011) formulated method to classify seed shape based on sphericity of the seed. As the sphericity based study used diameter of the greatest inscribed circle drawn from the seeds. This approach can overestimate the roundedness, as bold seeds could get more sphericity value though they are not round enough. In the present study, classification criteria solely based on seed shape was developed by including characteristics of chalazal and micropylar/radicle ends. The present study envisages the development of feasible seed shape classification system on the basis of seed components or appendages. This classification system will leads to easy and reliable phenotyping and also helpful for selection for other seed attributes.

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How to cite this article: Sivakumar, K.B., Gautam, A., Singh, S., Panwar, R.K., Arora, A. and Verma, S.K. (2023). A Pragmatic Study on Seed Shape Classification and its Association among Seed Quality Attributes in Chickpea (*Cicer arietinum* L.). Legume Research. doi:10.18805/LR-5025.

Submitted: 08-08-2022 **Accepted:** 30-05-2023 **Online**: 20-07-2023

MATERIALS AND METHODS

Experimental material and field trial

The field experiments of present study were conducted during the *rabi* seasons of 2018-19 and 2019-20 at the N.E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand. Seven diverse chickpea genotypes including angular, owl-head and globular types of seed shapes were selected and crossed in all possible combinations in a half diallel design. The material used for crossing included PG 3 a desi owl-headed type, PKG 2 a kabuli owl-headed type, three angular types *viz.,* PG 4, PG 5 and PG 170 and two globular types *viz.,* T 39-1 and T 39-1 A. Observations on seed shape were also recorded on five varieties *viz.,* PG 114, PG 158, PG 172, PG 186, PKG 1.

Observations on seed shape

The parents and F_{1S} were subjected to seed shape observations based on their radicle and cotyledonary portions. A total of 150 plants were observed for the seed shape. The F_{1S} which showed distinct classes for seed shape were observed and categorized in three classes as angular, owl-head and pea types. The seed shape of parental types and their respective F_{1S} were compared and the observations on dominance of seed shape were recorded. Based on the relative proportion of radicle (beak portion), the cotyledonary boundaries and curvature, the seed shapes were observed into three categories as angular, Owl-head and globular/pea types. The angular types had an elongated beak portion. On the chalazal end, a slight depression was observed. Owl headed types had a distinct, prominent Owl-head like beak portion with adjoining cotyledon region. Similarly, the cotyledons were protruding outwards at the chalazal end of the seed, which gave the Owl-head seed shape. There were no specific cotyledonary protrusions found at the chalazal end.

Estimation of protein content

The seed protein content was estimated as per kjeldahl method in the food testing laboratory of Department of Food Science and Technology, G. B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand, India. The protein content was estimated in all the parental and F_1 seeds of every cross combinations. Protein content was estimated from randomly selected seeds from each entry and carried out for two replications (Kirk, 1950).

RESULTS AND DISCUSSION

The observations based on the actual shape (proportion of the beak portion and the boundaries) and topologies of cotyledon region were considered as a criterion to portrait the seed shape. Fig 1 and 2 depicts the representative shapes of seed shape. The proportion of the radicle beak to the cotyledonary region was high in case of angular type and least in case of globular type. In the Owl-head type, the proportion is intermediate.

Perusals of Table 1 indicated that globular shaped seeds had round/flat chalazal end or abaxial end (when considering the radicle end on adaxial side). Angular types had slightly indented curvature on chalazal end, whereas owl headed types had deep indentation. This deep indentation is the base for the characteristic Owl-head shaped appearance. The radicle portion of globular seed was short or negligible in length, whereas it was long and intermediate in case of angular and owl-head type respectively. Globular types were

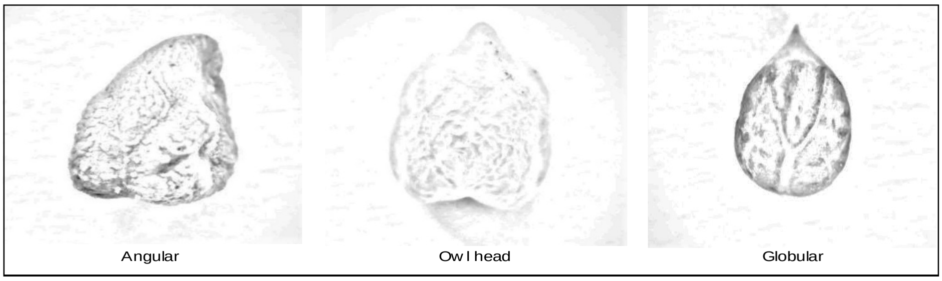

Fig 1: Diagrammatic representation of Angular, Owl-head and Globular shaped seeds.

Table 1: Specifications of seed characters among various seed shapes of 7 parents and their 14 crosses.

Parental types	Chalazal end	Beak (radicle portion)	Seed texture
Globular	R/F	S	S
Angular	Si	L	C
Owl-head	Di	M	C
Globular × Globular	R/F	S	S
Angular × Angular	Si	L	C
Owl-head desi × Owl-head kabuli	Di	M	C
Angular × Owl-head	Si	M*	C
Globular × Owl-head desi	F/R	M	S
Globular × Owl-head kabuli	F/R	M	S
Globular × Angular	Si	M*	C

Chalazal end: F- Flat, R- Round, Si- Slightly indented, Di- Deeply indented; Beak: L- Long, M- Medium S- Short

Texture: S- Smooth, C- Coarse.

*Radicle portion with medium length, yet doesn't resemble the characteristic Owl-head shape.

smooth textured. *i.e.,* no specific demarking undulations have found on the seed surface. Angular and owl-head types generally had coarse seed texture.

Parental types were classified as angular, globular and owl head types. Each of them had distinct characteristics in radicle portion, chalazal and overall shape. Where the F_{1s} has dominance for these components in one or other combination. There were no deviations in the seed shape characters when owl-head shaped kabuli types were crossed with desi owl head types, they incurred same effect on the seed shape characters except seed coat colour. A hybrid between PKG 2 (kabuli type) and PG 3 (desi type), essentially similar to their seed shape characters like shape of chalazal end and shape of radicle end, except white coloured maculation in cotyledonary area (Fig 5). Hybrids between two desi owl head types showed no changes in seed shape in comparison to their parents. Shape of the chalazal end showed dominance effect where as length of the radicle was shown to resemble co-dominance as there all the crosses between extreme types produce intermediate length radicle.

Dominance effect on shape of chalazal end and length of radicle end

Angular x Owl-head crosses and Angular x Globular crosses produced slightly indented chalazal end which was an attribute of angular type. Similarly, Globular x Owl-head type produced flat/round chalazal end, which was attributed to globular type. Hence, for the shape of chalazal end concern, angular types were dominant over Owl-head and globular types, where globular types were dominant over Owl-head types. The perusals of Table 2 indicated the dominance relationship which can be given as follows:
Angular > Globular >Owl-head.

The diagrammatic representation of beak shape and chalazal end shape in three classes of seeds were given in Fig 3 and Fig 4 respectively. The radicle length always exhibited a sort of intermediate values when extreme types were crossed. The cross between angular type (long radicle beak) and globular type (short or no prominent beak), always produces the seed with intermediate length. Angular × Owl-head and Globular × Angular also produced seeds with intermediate radicle, but not having the characteristic Owl-

Table 2: Dominance relationship of seed shape in chickpea.

Seed shape of parents	Seed shape of F1s
Angular × Globular	Angular
Angular × Owl-head desi	Angular
Angular × Owl-head kabuli	Angular
Globular × Owl-head desi	Globular
Globular × Owl-head kabuli	Globular

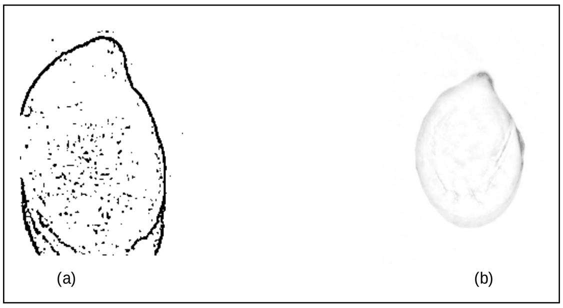

Fig 2: Diagrammatic representation of lateral view of Angular (a) and Globular (b) shaped seeds. The relative proportion of radicle and cotyledonary region can be clearly observed.

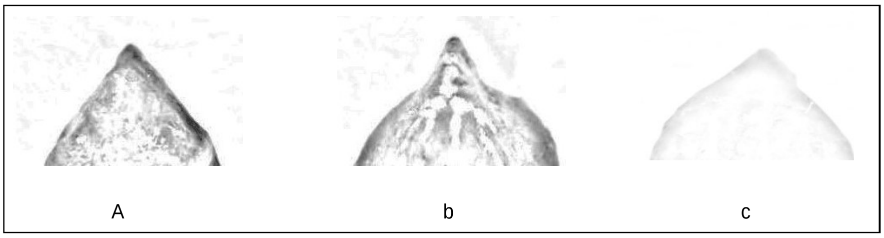

Fig 3: Representative shapes of radical /beak portion in angular (a), Owl-head (b) and globular (c) shaped chickpea seeds.

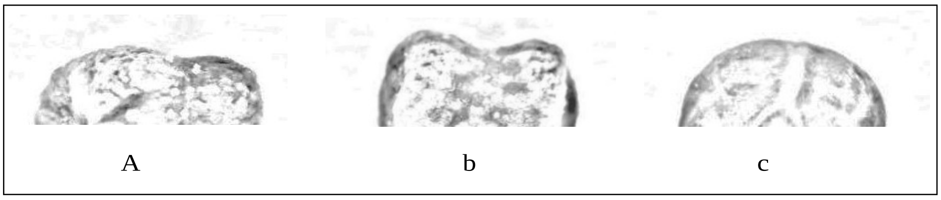

Fig 4: Representative shapes of chalazal end in angular (a), Owl-head (b) and globular (c) shaped chickpea seeds. Angular types showed slight indentation, Owl-head type showed deep indentation where globular types showed no indentation or flat.

head shape. While considering the seed texture, globular types were smooth textured where as angular and Owl-head types had distinguishable ridges and undulations on the seed surface. The progenies of the crosses angular x owl-head produced coarse textured seed. Crosses between Globular and Owl-head tend to produce progenies with smooth texture as of Globular type. From the crosses between extreme types (Angular × Globular), it was observed that coarse seed coat is dominant over smooth one. Pictorial representations are given in Fig 5.

Based on the above-said seed shape parameters, the overall dominance effect of seed shape can be plausibly described. Angular type was considered to be dominant over both Owl-head and globular types. The globular type was recessive to angular type but dominant over Owl-head type.

Considering the above criteria, the angular and round types were assumed as extreme types and owl-head as intermediate type. By crossing owl-head type with angular, or owl-head with globular, the progenies were showed to have extreme types, *i.e* angular and round respectively. Now, this is apparent that, while crossing primitive type with evolved variant, the dominance of ancestral allele might observe. Here, owl-head type, supposed to be an evolved variant get suppressed or hypostatic to ancestral / primitive (round and angular) type. As most of the kabuli genotypes are owl-headed types, it's quite clear that kabuli types are

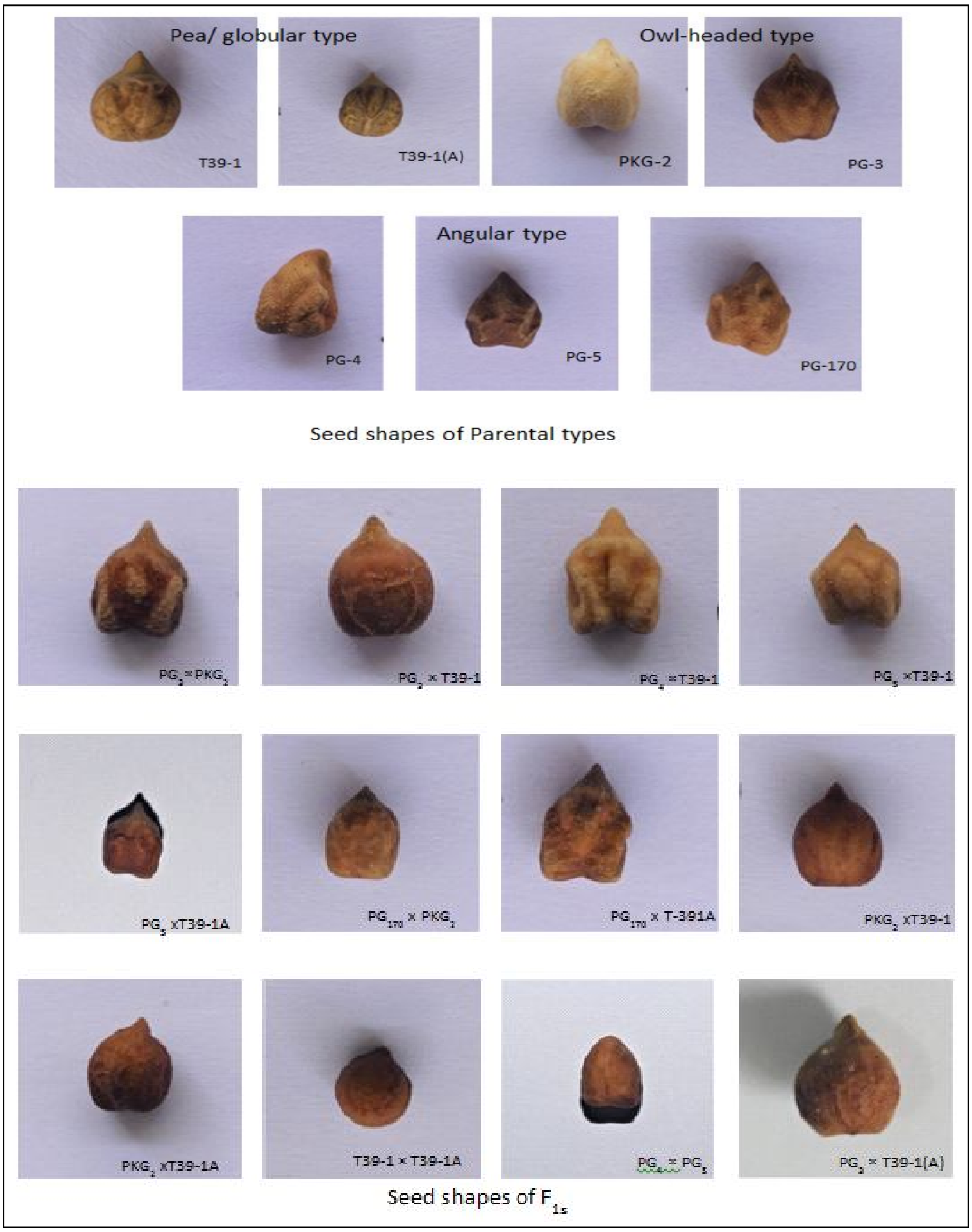

Fig 5 Pictures of parental and F1 seeds depicts the seed shapes.

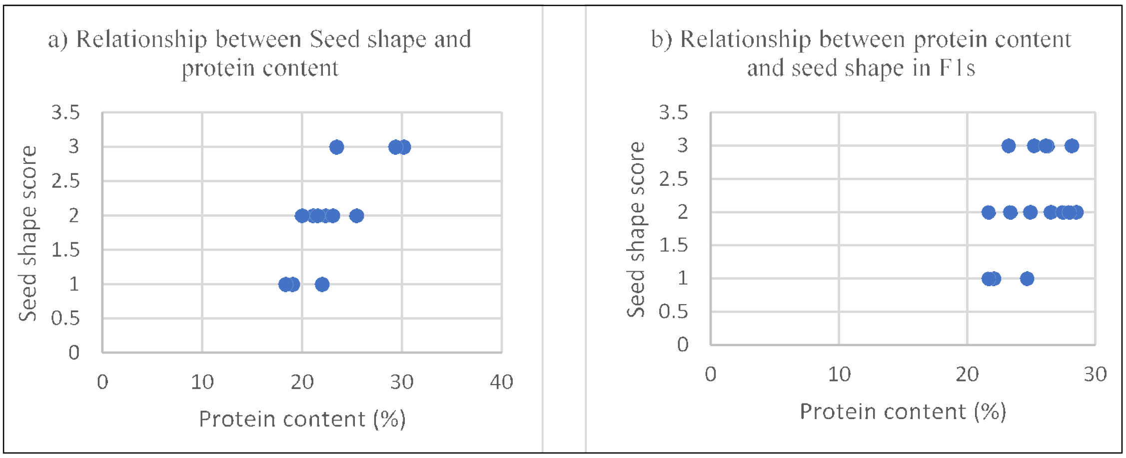

Fig 6: a) Correlation between seed shape and protein content among 12 varieties (a) and 15 F1s from crosses between selected parents (b). The seed shape score has been given based on roundedness as 1- angular, 2- Owl-head, 3- globular/pea

mutants evolved from the desi /ancestral types. Similar conclusions were also drawn in mutation experiments previously by Toker (2009).

Implications of seed shape on protein content

Chickpea is an important source of quality protein in vegetarian diets (Vijayalakshmi *et al.,* 2001). As seed shape is considered to be a qualitative trait, scale has been provided for scoring three types of seed shapes. In the present study, the seed shape and protein content was subjected to correlation analysis. These two characters were observed to highly correlated in parental types, the pea shaped seed tend to have high protein content, which was followed by owl-head types and angular types. Correlation analysis using spearman's rank correlation were performed between average protein content and seed shape score among 12 genotypes which showed that, these two traits were highly correlated (r = 0.78**, significant at 1% level of significance, p value= 0.002). The correlation between these two traits weakened slightly in F1s (r= 0.4). The Scatter plot represents the relationship as given in Fig 6.

When analysing the F_{1s} for correlation, moderate correlation was recorded for these traits. The positive relationship between these two traits weakened in $F_{1s.}$ The possible reason may be that, protein content always produced intermediate values when crossing two extreme parents, still for moderate correlation (r= 0.4). Thus low protein content was found in the angular genotypes while Owl-head and globular types were indirectly correlated with high protein content. The protein content is found to be low to moderate in owl-head types, whereas round seeded types had relatively high protein content. For instance, PKG 1, a little round type had less seed weight than the owl-head variety PKG 2.

Implications of seed shape on seed weight

The 100 seed weight is quantitative measure of seed size and it is a major determining factor of other seed traits. By analysing the correlation among 100 seed weight and seed shape of some released varieties, which are angular and owl-headed types, showed high correlation over the other. The correlation analysis among 12 varieties showed high positive correlation (r = 0.7) for this trait. The globular shaped breeding lines had very small seed size, leading to less 100 seed weight (less than 10 g). But crosses between owl-head and globular types varieties produced F_1s with globular seed shape and high 100 seed weight similar to the owl-head types, though, several globular, bold typed seeds with high 100 seed weight could be observed in later segregating generations. Thus, these two traits could be manipulated with ease by developing large segregating populations. Such globular types would be a key to merge the bidirectionally acting seed traits like protein content and seed weight.

Thus in present study significant positive correlation was found among the seed shape, seed protein content and seed weight. Globular and owlhead seed types obtained from the crosses are having higher 100 seed weight and protein content. By utilizing this seed shape classification system, breeder can indirectly look for a segregant with higher seed weight and protein content. Hence, the current study may serve as a plausible approach, when considering the seed shape as a selection criteria, could make the breeding for yield and quality attributes more effective. Similarly the relationship between seed weight and protein content could be manipulated if we include seed shape as a bridging trait. Further studies on utilizing seed shape as an indexing trait to select desirable seed attributes will improve the selection process in plant breeding programmes. This study establishes a simple yet reliable classification system for seed shape in chickpea, which could be exploited to breed better quality varieties.

CONCLUSION

Globular seed form is small in size, have high protein content and have less 100 seed weight but when crossed with owl head types produced globular F_1s with more 100 seed weight. Thus, globular types would be a desirable trait to merge the bi-directionally acting seed traits like protein content and seed weight. Hence, the seed shape could act as the needed key for combining the major seed traits and potentially may emerge as the plausible selection criteria in future chickpea breeding.

ACKNOWLEDGEMENT

The authors are highly thankful to the Department of Genetics and Plant breeding, Joint director CRC and Director Experiment Station, GBPUA&T, Pantnagar for providing the necessary resources for the present study.

Contribution of authors

Ravindra Kumar Panwar came up with the concept and designed the experiments. The manuscript was written by Karthick Babu Sivakumar. Karthick Babu Sivakumar, Satvinder Singh and Ravindra Kumar Panwa carried out the experiments. Karthick Babu Sivakumar, Ravindra Kumar Panwar, Sanjay Kumar Verma and Ashish Gautam analysed the data. The data and manuscript were finalized by Karthick Babu Sivakumar, Ravindra Kumar Panwar, Ashish Gautam, Satvinder Singh, Sanjay Kumar Verma and Anju Arora. The final manuscript has been read and approved by all authors.

Conflicts of interest

The authors declare no conflict of interest.

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