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Inheritance of protein content and its relationships with seed size, grain yield and other traits in chickpea

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Abstract Chickpea (*Cicer arietinum* L.), the second largest grown pulse crop of the world, is an important source of protein for millions of people, particularly in South Asia. Development of chickpea cultivars with further enhanced levels of protein is highly desired. This study was aimed at understanding the genetic control of protein content and its association with other traits so that suitable breeding strategies can be prepared for development of high protein content cultivars. A high protein (29.2 %) desi chickpea line ICC 5912 with pea-shaped small seed, grey seed coat and blue flower was crossed with a low protein (20.5 %) kabuli line ICC 17109 with owl's head shaped large seed, beige seed coat, and white flower. The F_2 population was evaluated under field conditions and observations were recorded on protein content and other traits on individual plants. The protein content of F_2 segregants showed continuous distribution suggesting that it is a quantitative trait controlled by multiple genes. The blue flowered segregants had pea shaped seed with grey seed coat, while the white flowered segregants had owl's head shaped seed with beige seed coat indicating pleiotropic effects of gene(s) on these traits. On an average, blue flowered segregants had smaller seed, lower grain yield per plant and higher protein content than the pink flowered and the white flowered segregants. The protein content was negatively correlated with seed size ($r = -0.40$) and grain yield per plant ($r = -0.18$). Thus, an increment in protein content is expected to have a negative effect on seed size and grain yield. However, careful selection of transgressive segregants with high protein content along with moderate seed size and utilizing diverse sources of high protein content will be usefull in developing chickpea cultivars with high protein content and high grain yield.

Keywords Chickpea · *Cicer arietinum* · Correlations · Flower color · Inheritance · Protein content · Seed size

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Introduction

Chickpea (*Cicer arietinum* L.) is the world's second largest grown pulse crop after beans and is mainly

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used for human consumption. During 2013, the global chickpea production reached a record high of 13.1 million metric tons with 84.5 % of the production coming from Asia (75.5 % from Southern, 5.0 % from Western and 3.7 % from South-Eastern), 6.2 % from Oceania, 4.6 % from Americas (2.5 from Northern, 1.6 from Central and 0.5 % from Southern), 4.1 % from Africa (3.4 % from Eastern and 0.6 % from Northern) and 0.7 % from Europe (0.4 % from Eastern and 0.3 % from Southern) (FAOSTAT 2015). There is an increasing awareness of the nutritive value and health benefits of chickpea. The global chickpea production has increased by 56 % during the past decade (2004–2013). Among the major chickpea producing countries, chickpea production increased by 55 % in India, 502 % in Australia, 23 % in Pakistan, 118 % in Myanmar, 53 % in Ethiopia, 100 % in Mexico, 231 % in Canada, 485 % in USA, 275 % in Tanzania and 112 % in Malawi.

Chickpea constitutes an important source of protein in the diets of millions of people, particularly in South and Southeast Asia region which accounts for nearly 80 % of the global chickpea production and consumption. In addition to protein, chickpea is a good source of carbohydrates, dietary fibre, minerals (molybdenum, manganese, copper, phosphorus, iron and zinc) and vitamins (riboflavin, niacin, thiamin, folate and the vitamin A precursor beta-carotene) (Jukanti et al. 2012). The in vitro protein digestibility of chickpea seeds was found to be higher compared with those for pigeonpea, mungbean, urdbean and soybean (Chitra et al. 1995). Chickpea is generally consumed in combination with cereals (wheat, rice and maize) and the mixed diet provides all the essential amino acids by complementing each other for limiting amino acids (lysine in cereals and sulphur-containing amino acids in chickpea). Chickpea consumption is known to have potential beneficial effects on lowering risk of some of the important human diseases such as cardio vascular diseases, type 2 diabetes, digestive diseases and some types of cancers (Jukanti et al. 2012).

There are two distinct types in chickpea, desi and kabuli. The desi types have thick and coloured (mostly brown) seed coat, while the kabuli types have thin and cream-coloured seed coat. The desi types account for about 80–85 % of the global chickpea area and are largely grown in South Asia, Eastern Africa, and Australia and mainly consumed in South Asia (Gaur et al. 2015). Seed coat contains 80 % of the crude fiber and all the anti-nutritional polyphenols (Singh et al. 1980) and plays an important role in nutritive value, cooking time and processing quality of chickpea. The mean seed coat dietary fibre content was reportedly 14.2 % in desi type and 4.9 % in kabuli type (Singh et al. 1980). The desi types have a higher total dietary fibre content and insoluble dietary fibre content compared with the kabuli types due to thicker seed coat (Rincon et al. 1998). The kabuli types were found to have higher hydration and swelling capacity than the desi genotypes (Tripathi et al. 2012). The in vitro digestibility of protein from the kabuli types was found to be higher than that from the desi types (Sanchez-Vioque et al. 1999; Paredes-Lopez et al. 1991).

The protein content of currently available chickpea cultivars generally ranges between 20 and 22 %, while a wide range of variation, from 12 to 30 %, exists in chickpea germplasm (Pundir et al. 1988; Jadhav et al. 2015). Thus, it seems feasible to develop cultivars with 20–25 % higher protein content than the existing cultivars. However, there have been limited breeding efforts on further enhancing protein content in chickpea.

Information on inheritance pattern and relationships of protein content with other traits would help in identifying suitable breeding strategies for developing chickpea cultivars with enhanced protein content, high yield, market preferred grain traits (size, shape and color), and other desired agronomic traits. Thus, this study was conducted to study inheritance of protein content in an F_2 population and the relationships of protein content with other traits.

Materials and methods

Experimental materials

Two chickpea genotypes (ICC 5912 and ICC 17109) showing variability for flower color, protein content, seed coat color and 100 seed weight (Table 1) were crossed during summer season, 2008 under glasshouse conditions. True F_1s were grown in post-rainy season 2008/09 for generation advancement. F_2 population along with the parental lines were grown during post-rainy season 2009/2010 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India. Seeds were planted on 4 m long ridges at a spacing of 30 × 10 cm under normal field conditions. Two irrigations

Table 1 Differences between the parental genotypes ICC 5912 and ICC 17109 for selected qualitative and quantitative traits

Traits	ICC 5912	ICC 17109
Flower colour	Blue	White
Seed coat colour	Grey	Beige
Seed shape	Pea	Owl's head
Plant height (cm)	40.7 ± 1.6	54.6 ± 1.94
Days to maturity (days)	122 ± 0.3	118 ± 0.34
Seed number/plant	28 ± 7.1	27 ± 4.32
Seed yield (g)	2.8 ± 0.7	13.9 ± 2.14
100-seed weight (g)	10.2	54.5
Protein content (%)	29.2	20.5

were provided—one during vegetative phase and another at pod filling stage. Necessary disease and pest management practices were followed to raise a healthy crop.

Experimental observations and protein estimation procedure

Observations were taken on flower colour, days to flowering, days to maturity, plant height (cm), number of seeds, and grain yield on 10 randomly selected plants from each parental line and all 265 F_2 segregants. Freshly opened flowers were used for recording flower color and flowering time. Data on number of seeds per plant and grain yield per plant were used for calculating 100-seed weight (g). Seed coat color of F_2 derived F_3 seeds was classified into brown (177B), beige (165D) and and gray (196A) based on Royal Horticultural Society color chart (http://cropgenebank.sgrp.cgiar.org/images/file/management/chickpea.pdf). Seed shape was visually classified into two classes, pea shape and owl's head shape.

Protein content of grains harvested from each plant was estimated by using standardized, automated colorimetric procedure through the estimation of nitrogen using a single digest (sulfuric acid selenium digestion) method. This method uses sulfuric acid containing selenium (Se) to digest and prepare plant materials for nitrogen analysis in the same digest. Approximately 0.5 g of finely ground seed sample was transferred to 250 ml digestion tubes. Fourteen ml of concentrated sulfuric acid containing 0.5 % selenium (by wt. as metal) powder was added to soak the seed material held in each tube. Sulfuric acid and Se mixture was prepared by dissolving Se powder in concentrated sulfuric acid by heating on a hot plate with occasional mixing by stirring with a glass rod. Five grams of Se powder was added to about 500 ml of sulfuric acid and heated to dissolve the Se powder. The mixture was cooled and after adding the digestion mixture to plant materials, digestion tubes were transferred to a block digester preheated to 370 °C. Nearly 2.5 h were needed for completing the digestion, indicated by clear and colorless plant digests. The digests were adjusted to 250 ml by adding distilled water. The suitable aliquots of digests were used to determine nitrogen by distillation with sodium hydroxide, using an atomic absorption spectrophotometer (Jones et al. 1991; Sahrawat et al. 2002). Protein content (composed as %) was obtained by multiplying the total nitrogen content in the seeds by the multiple factor 6.25 (Jones 1941).

The data were analyzed using Windostat software package, for testing appropriate F_2 phenotypic ratios (χ^2) and studying mean differences (t test) among different segregating groups for various traits. Phenotypic correlations were calculated between protein content and yield related traits. Normal distribution of different data sets was estimated using Shapiro–Wilk test in excel.

Results and discussion

The two parental lines, ICC 5912 and ICC 17109, differed significantly for several qualitative and quantitative traits (Table 1). The desi chickpea line ICC 5912 had blue flowers, grey seed coat, pea shaped seed, small seed (10.2 g 100-seed^{-1}), low grain yield (2.8 g plant^{-1}), and high protein content (29.2 %), while the kabuli chickpea line ICC 17109 had white flowers, beige seed coat, owl's head shaped seed, large seed (54.5 g 100-seed^{-1}), high grain yield (13.9 g plant^{-1}) and low protein content (20.5 %). As compared to ICC 5912, ICC 17109 was taller and early maturing. Thus, the F_2 poulation derived from ICC 5912 × ICC 17109 segregated for several traits.

Inheritance of protein content and other traits

The frequency distribution in F_2 population appeared as continuous variation for protein content and 100-seed weight (Fig. 1). However, the calculated Shapiro–Wilk statistic W for protein content (0.985)

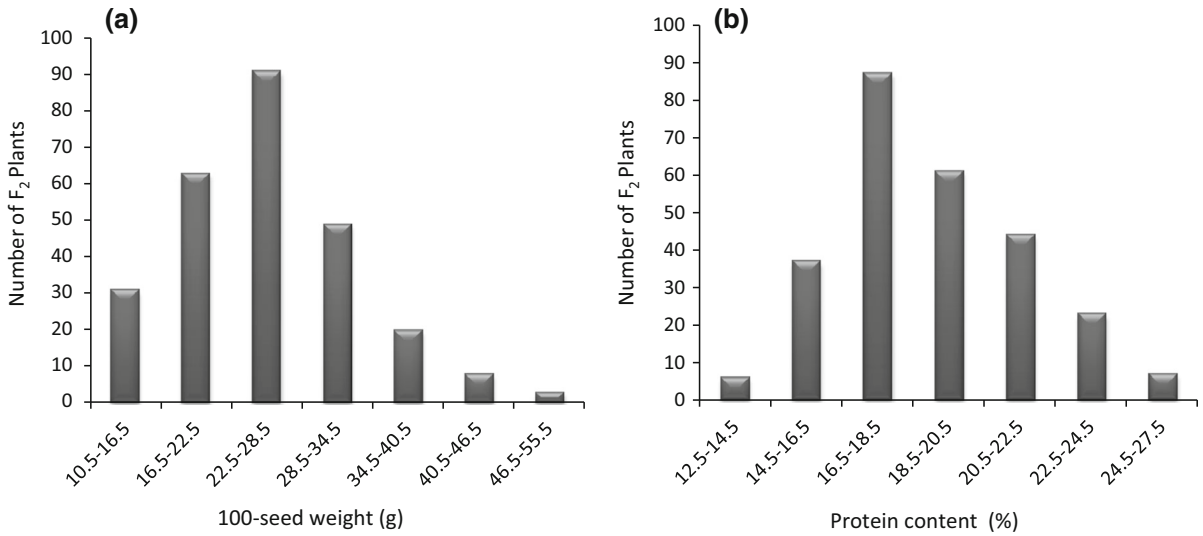

Fig. 1 Frequency distribution for (**a**) 100-seed weight and (**b**) protein content in F_2 population of chickpea cross ICC 5912 × ICC 17109

and 100-seed weight (0.970) was higher than the crital value of W (0.947) at 5 % significance level and thus the null hypothesis was rejected. This indicates qualitative inheritance and oligogenic control of these traits. However, the normal frequency distribution for protein content in F_2 population of chickpea was reported by Vijayalakshmi et al. (2001). There are variable reports on the inheritance of seed size in chickpea, mainly because of the differences in the parents used. These include control of seed size by a single gene (Argikar 1956), two genes (Ghatge 1993; Upadhyaya et al. 2006; Hossain et al. 2010) and polygenes (Malhotra et al. 1997). Normal distribution of data for 100-seed weight was also reported in F_2 population, where ICC 17109 was used as one of the parents in a cross (Sharma et al. 2013).

Studies have been conducted to identify molecular markers linked to quantitative trait loci (QTLs) controlling protein content and 100-seed weight (seed size) in chickpea. Association mapping studies on 187 genotypes identified four QTLs for protein content, and the amount of variation explained by these marker trait associations (MTAs) ranged from 2.4 to 5.1 % (Jadhav et al. 2015). These MTAs for protein content need to be validated in different populations of chickpea for use in regular breeding programs. Bi-parental mapping populations developed from the crosses between desi and kabuli chickpeas have been used for molecular mapping of QTLs for 100-seed weight. Two QTLs showing phenotypic variance of 30 % (Cobos et al. 2007) and 37 % (Hossain et al. 2010) have been reported for 100-seed weight. However MTAs for protein content and seed size 100-seed weight were not evaluated in the present study.

The F_1 from ICC 5912 (blue flowers) × ICC 17109 (white flowers) produced pink flowers. In F_2, flower colour segregated in a ratio of 9 (pink): 3 (blue): 4 (white) suggesting that this trait is controlled by recessive epistatic interactions between two genes (Table 2). These results confirm the previous findings on inheritance of flower colour in chickpea (Gaur and Gour 2001).

The seed shape and seed coat color of F_1 were studied on F_2 seeds and of F_2 segregants on F_3 seeds. The F_1 gave pea shaped seed suggesting dominance of pea seed shape on owl's head seed shape. The F_2 population gave a good fit to a 3 (pea): 1 (owl's head) ratio (χ^2 value of 0.02; $P = 0.887$) (Table 2) which suggests that this trait is controlled by a single gene. Similar results were obtained earlier from crosses between genotypes with pea shaped and owl's head shaped seeds (Kumar et al. 1985; Meena et al. 2004) and from crosses between genotypes with pea shaped and angular seeds (Knights et al. 2011).

The seed coat color of F_1 was brown and the F_2 population segregated in a ratio of 9 brown: 3 grey: 4 beige ($\chi^2 = 0.889$; $P = 0.641$) (Table 2) suggesting recessive epistatic control of seed coat color in this

Table 2 χ^2 test for F_2 segregation of flower colour, seed coat colour and seed shape in chickpea cross ICC 5912 × ICC 17109

Traits	Phenotypic class	No. of plants	Total	Phenotypic ratio	χ^2 value	P value
Flower colour	Pink	156	265	9:3:4	0.889	0.641
	Blue	45				
	White	64				
Seed coat colour	Brown	156	265	9:3:4	0.889	0.641
	Grey	45				
	Beige	64				
Seed shape	Pea	200	265	3:1	0.020	0.887
	Owl's head	65				

cross. There are variable reports on the inheritance of seed coat color in chickpea with respect to the number of genes segregating in a cross and interactions of these genes. Monogenic (D'Cruz and Tendulkar 1970), digenic (Bhapkar and Patil 1963; More 1976; Pawar and Patil 1979), tetragenic (Alam 1935) and pentagenic (Ayyar and Balasubramanian 1936; Brar and Athwal 1970) inheritance with different gene actions have been reported for seed coat colour in chickpea. Variations in partitioning seed coat colors into different classes may also contribute to the differences in observed results.

The F_2 plants were classified into different categories based on qualitative traits (flower color, seed coat color, seed shape). All blue flowered segregants had pea shaped seed with grey colored seed coat, while all white-flowered segregants had owl's had shaped seed with beige colored seed coat. The pink flowered plants had pea shaped seed with brown colored seed coat of varying color intensities. These results suggest pleiotropic effects of gene(s) on these traits.

The blue flowered segregants had on an average smaller seeds (16.21 g 100-seed^{-1}) and higher protein content (21.81 %) than the pink flowered (25.95 g 100-seed^{-1}; 18.33 % protein) and the white flowered segregants (30.68 g 100-seed^{-1}; 18.89 % protein) (Table 3). Significance of the differences in protein content and 100-seed weight between different categories of plants was tested using *t*-test (Table 4). There were significant differences in protein content as well as 100-seed weight between the plant categories pink vs blue flowers, blue vs white flowers, brown vs grey seed coat and grey vs beige seed coat. The plant categories pink vs white flowers, brown vs beige seed coat, and pea vs owl's head seed shape showed significant difference for 100-seed weight and did not differ significantly for protein content. The coefficient of determination R^2 value was higher between protein content and 100-seed weight ($R^2 = 0.16$) than between protein content and seed yield ($R^2 = 0.03$). Nonetheless, there were some F_2 segregants with high protein content and moderate 100- seed weight and high grain yield (Fig. 2).

The high protein content in blue flowered, grey seeded segregants could be because of their reduced seed size compared to pink flowered and white flowered segregants. Kumar et al. (1982) also found that blue flowered plants with smaller seeds had higher protein content compared to pink flowered plants with larger seeds. They suggested linkages between genes for flower colour, protein content and seed weight. Existence of linkage between flower colour and seed size was also reported by Atta et al. (2003). However, protein content in pink and white flowered classes did not show any significant difference in our study.

Correlation coefficients were computed between protein content, days to maturity, plant height, number of seeds per plant, grain yield per plant, and 100-seed weight (Table 5). Correlation between protein content and plant height was positive and significant (0.30**). Similarly, a significant positive correlation was found between protein content and days to maturity (0.14*). These results are similar to the findings in soybean where significant positive correlations of protein content with days to maturity and plant height were found (Bellaloui et al. 2009; Copur et al. 2009). Negative and significant correlations were found between protein content and 100-seed weight (−0.40**) and protein content and grain yield (−0.18**). A negative relationship between seed size and protein content implies that as seed increases in size there is an increased amount of starch deposition altering the starch/protein

Table 3 Mean and range along with standard error for protein content and 100-seed weight in different phenotypic classes of F_2 in chickpea cross ICC 5912 × ICC 17109

Trait	Phenotype	Protein content (%)		100-seed weight (g)	
		Mean	Range	Mean	Range
Flower color	Pink	18.33 ± 0.19	12.84–26.72	25.95 ± 0.47	11.61–55.00
	Blue	21.81 ± 0.34	17.10–26.08	16.21 ± 0.47	10.83–24.72
	White	18.89 ± 0.27	14.77–24.10	30.68 ± 0.93	19.39–52.94
Seed coat colour	Brown	18.33 ± 0.19	12.84–26.72	25.95 ± 0.47	11.61–55.00
	Grey	21.81 ± 0.34	17.10–26.08	16.21 ± 0.47	10.83–24.72
	Beige	18.89 ± 0.27	14.77–24.10	30.68 ± 0.93	19.39–52.94
Seed shape	Pea	19.09 ± 0.20	12.94–26.72	23.75 ± 0.48	10.83–55.00
	Owl's head	18.91 ± 0.27	14.77–24.10	30.70 ± 0.92	19.39–52.94

Table 4 Means comparisions for 100-seed weight and protein content between different phenotypic classes of F_2 in chickpea cross ICC 5912 × ICC 17109

Trait	Class (number of F_2 plants)	T-value (two sample unequal variances)	
		100-seed weight	Protein content
Flower colour	Pink (156)-Blue (45)	14.67**	−8.86**
	Pink (156)-White (64)	−4.54**	-1.69^{NS}
	Blue (45)-White (64)	−13.86**	6.71**
Seed coat colour	Brown (156)-Grey(45)	14.67**	−8.86**
	Brown (156)-Beige (64)	−4.54**	-1.69^{NS}
	Grey (45)-Beige (64)	−3.86**	6.71**
Seed shape	Pea (200)-Owl's head (65)	−6.64**	0.35^{NS}

NS non significant

*, ** Significant at <0.05 and <0.01 probability level

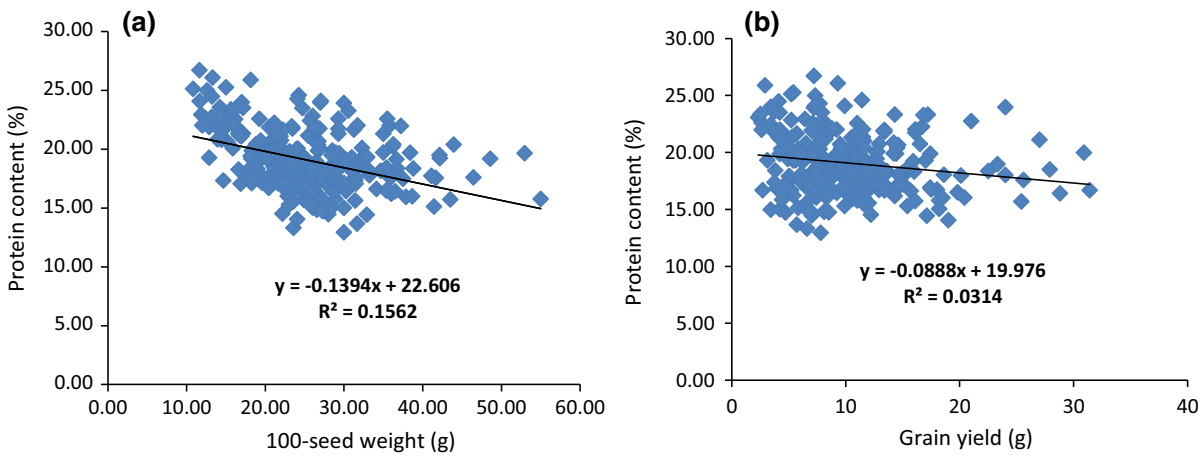

Fig. 2 Relationship between (**a**) protein content and 100-seed weight and (**b**) protein content and grain yield in F_2 population of chickpea cross ICC 5912 × ICC 17109

ratio (Bahl et al. 1979). Protein content and starch content have been found to be negatively correlated in chickpea (Frimpong et al. 2009; Gangola et al. 2012). Similar to this study, protein content was found to be negatively correlated with seed size in pigeonpea. However, breeding lines combining high protein content with medium seed size were successfully developed (Saxena et al. 1987).

The existence of a negative correlation between protein content and seed size and significant associations of protein content with flower color, seed coat color and seed shape suggests that development of

Table 5 Phenotypic correlation coefficients between protein content and various yield component traits in F_2 of chickpea cross ICC 5912 × ICC 17109

Traits	Plant height (cm)	Seed number	100-seed weight (g)	Seed yield (g)	Protein content (%)
Days to maturity	0.09	0.03	−0.07	−0.01	0.14*
Plant height (cm)	–	0.13*	0.08	0.18**	0.30**
Seed number		–	−0.35**	0.84**	0.06
100-seed weight (g)			–	0.13*	−0.40**
Seed yield (g)				–	−0.18**

*, ** Significant at <0.05 and <0.01 probability level

chickpea cultivars with high protein content and desired seed traits (size, shape and color) would require large segregating populations and the selection of desired recombinants. Blue flower color, grey seed coat color and pea seed shape of high protein line ICC 5912 showed pleiotropic effects of gene(s) and these traits were also associated with seed size and protein content. Thus, it would be important to search for other high protein lines in the germplasm and use diverse sources of high protein content in breeding programs for development of high protein chickpea cultivars with desired seed traits (size, shape and color).

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