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HERITABLE VARIATION AND CORRELATION BETWEEN SEED SIZE, STARCH CONTENT AND ITS COMPOSITION IN CHICKPEA

A. BAKHSH, C.L. HEDLEY* B.A. MALIK, S.M. IQBAL AND M. ARSHAD

Pulses program National Agricultural Research Centre, Islamabad, Pakistan.

Abstract

A study was undertaken to estimate correlation coefficient between seed size, starch content and its composition together with the estimation of genetic variability in a range of chickpea genotypes. Among the 15 chickpea genotypes of diverse origin analyzed highly significant differences which ranged from 191 mg to 496 mg were found for weight per seed. Relatively small differences from 38-45% of seed dry weight were found for total starch whereas values for amylose and amylopectin percentage of starch varied from 32-42% and 58-68% respectively. Within genotype variation for starch content and amylose percentage was insignificant which could be due to experimental error. However, there were considerable differences within genotype for seed size in almost all the lines. The correlation between seed size and starch content and composition was negligible although positive and there was no association between starch percentage and amylose percentage of starch.

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop in Pakistan pimarily grown as a source of vegetable protein. The seeds contain upto 47% starch and 30% protein of the seed dry weight (Singh, 1985). The seed size and seed yield is therefore, primarily dependent upon the amount of these storage products deposited during seed development.

A considerable variation in the content of chickpea starch has been reported by Sambunathan & Singh (1980) and Singh (1985), which may range from 41-51% of dry weight in desi and from 45-51% in kabuli chickpea. Amylose content of chickpea starch also varies from 20-30% of total starch (Singh, 1985). It has been reported that amylose percentage of starch in legumes may be influenced by environmental effects (Bhatty, 1988).

The genetics of starch and amylose has been well documented in maize (Kramer & Wistler, 1949; Camaron, 1947) and pea (Kellenbarger *et al.*, 1951; Wang & Hedley, 1991). High starch content in pea has been shown to be dominant over low, and low amylose to be dominant over high (Kellenbarger *et al*, 1951)

Following a study of corn endosperm starch, Kramer & Wistler (1949) suggested the possibility of association between high amylose and low starch. However in pea, Wang & Hedley (1991) reported that high amylose and low starch may not always be associated. In the present study a correlation between seed size, starch (percentage of dry weight) and amylose percentage of starch together with the assessment of the level of variation for these parameters in chickpea is presented.

*John Innes Research Institute (JII) of Plant Sciences, Norwich, U.K.

Materials and Methods

Plant material: A total number of 15 genotypes developed in Pakistan (1), ICRISAT, India (2) and ICARDA, Syria (12) were used. The genotypes were grown at the NARC, Islamabad during the rabi reason 1994 and their seeds harvested for chemical analysis.

Starch and amylose analysis: Five seeds were randomly selected from each of the 15 genotypes and weighed individually. All the selected seeds of each genotype were separately ground for 5-6 minutes, after removing their testa, in a shatter box grinder (Glen Creston mil). The flour samples were desiccated over P_2O_5 for at least 24 hours before their analysis. Total starch was analyzed using a procedure based on total enzyme digestion to glucose (Carpita & Kanabus, 1987). Duplicate samples of chickpea flour were weighed (5-6mg) in 15ml screw capped tubes and 1ml 90% dimethyl sulfoxide (DMSO) was added to each tube. The contents of the tubes were stirred overnight, using a magnetic stirrer, following an initial period of 2 h in boiling water. On the following morning, 4ml of a solution, prepared by adding 0.01ml α-amylase and 0.1ml of amyloglucosidase per 4ml of acetate buffer was added to each tube and the tubes were placed in a water bath for 2.5 h. For the first 30 minutes of incubation, the temperature of the water bath was maintained at 20^0C and then increased to 53^0C for the remaining 2 h. 5mls of GOD period solution was added to replicate 100 μl samples of extract. Glucose standards were prepared by adding 100 μl of glucose standard solution to 5ml of GOD period. The solutions were mixed using a vortex mixer. The tubes were placed in a water bath set at 38^0C for 30 minutes and after cooling the absorbance of each sample was measured using a spectrophotometer set at 578nm wave length. The absorbance readings were corrected to equate to a 5mg starting sample and an average of 4 readings was used to calculate the proportion of starch in the flour and the total starch using the following formulae:

$$\mu\text{g starch/ 5mg sample} = \frac{455 \times \text{Absorbance per 5 mg sample}}{\text{Absorbance of standard solution}}$$

$$\text{Percent starch} = \frac{\mu\text{g starch in 5 mg sample} \times 100}{5000}$$

Amylose content was determined using a chemical analytical method similar to that of Knutson (1986). After desiccation, duplicate 5 to 6mg samples of flour were weighed into screw capped tubes. Two ml of 85% methanol were added to each tube which were then left stirring for 2 h. The tubes were centrifuged for 15 minutes and the supernatant was drawn off gently without disturbing the pellet. The remaining methanol was evaporated by keeping the tubes in warm water for 1.5 h. To each sample, 10ml of a solution containing 90%DMSO + 0.006m iodine were added and the tubes placed in a cold water bath which was brought to boiling point for 2 h. The contents of the tubes were continuously stirred for at least 12 h. 0.5mls solution from each tube was taken and added to 8mls of distilled water and mixed using a vortex mixer. The tubes were

then left for 30 min., to allow the colour of solution to develop before recording absorbance at 600nm wave length using a spectrophotometer. Four readings for each sample were taken and the average absorbance was then recalculated for a 5mg sample. The percentage amylose was determined from a calibration curve, produced using standard amylose solutions. The percentage of amylose in starch was calculated using the following formula:

$$\text{Percent amylose in chickpea starch} = \frac{\text{Amylose in 5mg chickpea flour x 50 x 100}}{\mu\text{g starch in 5mg chickpea flour}}$$

Data regarding seed weight, starch content and amylose percentage of starch was separately recorded for each seed in all the accessions. The set of data on a single seed of a particular genotype were regarded as one replication. All the genotypes analyzed provided replicated data with 5 replications. The data was subjected to analysis of variance (Steel & Torrie, 1960) to determin the significance of differences between genotypes. Correlation Coefficients were computed following the method of Dewy & Lu (1959).

Results

Intra-genotypic Variation: The level of variation within the seed populations of individual genotypes showed that the seed size varied considerably in all the genotypes (Table 1) whereas total starch and its amylose percentage was almost similar in the seeds of a genotype.

Inter-genotypic variation: The results of analysis of variance indicated that there were significant differences for all the characters studied. The seed size in different genotypes ranged from 191mg to 496mg. However, means over the replications for this character were in the range of 291mg to 436mg whereas total starch, amylose and amylopectin varied from 38-45%, 32-42% and 58-68% respectively. Variety F88-83c showed minimum starch content and amylose percentage; with maximum starch found in Flip91-2C and amylose percentage in Flip84-124C (Table 2).

Correlation coefficients: The genotypic correlations of starch (0.31) and amylose (0.37) with seed size were non significant. Similarly, there was lack of genotypic correlation between amylose and starch (0.042). Values of phenotypic correlation coefficients of starch and amylose % with seed dry weight were 0.141 and 0.133, respectively whereas phenotypic correlation between amylose and starch was negligible (-0.052), although negative (Table 3).

Discussion

Before surveying genotypes for intergenotypic variability it is important to find out any differences which may be present within genotypes. Such differences are usually attributed to various environmental factors. However, the role of minor genes which may differ from plant to plant in a population can not be ruled out.

Table 1. Intragenotypic variation for seed size, starch, content in 15 chickpea genotypes.

	Range		
	Seed weight (mg)	Starch (%)	Amylose %age
CM-72	191-234	40.1 - 40.6 (0.37) (1.61)	30.3 - 34.1 (0.15) (2.20)
F88-83C	237-304	38.7 - 39.1 (1.6) (0.04)	30.7 - 35.0 (1.17) (2.08)
CA188220	358-441	39.8 - 38.7 (0.15) (0.51)	34.1 - 34.7 (0.37) (1.11)
CA188380	256-432	42.2 - 43.20 (0.41) (0.42)	32.5 - 33.4 (3.64) (1.12)
ILC-72	278-326	42.2 - 44.4 (0.23) (0.33)	35.7 - 36.0 (0.46) (0.34)
ILC-200	200-257	41.9 - 44.00 (0.25) (0.18)	34.3 - 35.2 (0.51) (2.91)
ILC-6090	232-348	39.0 - 40.5 (0.39) (0.31)	32.7 - 35.3 (0.73) (0.50)
FLIP84-86C	269-381	38.6 - 38.6 (0.58) (0.83)	37.4 - 40.1 (0.52) (0.50)
FLIP84-124C	251-336	39.9 - 44.8 (2.05) (0.52)	41.4 - 41.8 (1.95) (0.28)
FLIP95-58C	326-472	40.7 - 42.2 (0.21) (0.60)	32.00 - 35.2 (1.47) (0.60)
FLIP85-58/ Sp. WHITE	347-404	43.1 - 40.9 (0.21) (0.53)	33.5 - 36.00 (0.34) (0.15)
FLIP 85-58C /Sp. White	348-496	41.6 - 43.5 (0.38) (0.71)	36.4 - 36.5 (0.40) (0.40)
FLIP 90-95C	302-357	40.1 - 44.8 (1.04) (0.29)	36.6 - 37.5 (0.83) (0.08)
FLIP 91-2C	370-498	44.3 - 44.5 (0.41) (1.90)	31.9 - 34.8 (1.03) (0.20)
FLIP 91-62C	281-369	44.6 - 46.2 (0.40) (0.28)	34.8 - 36.1 (0.28) (0.68)

Figures given in parenthesis are SE of corresponding values.

Seed development is achieved as a result of source and sink activity. The variation in seed size observed within genotypes in the present investigation may be attributed to the limited supply of nutrients to the last developing seeds. The variation in developmental stages of different seeds is in turn attributed to acropetal flowering pattern in chickpea. The number of seeds per pod may also account for seed size differences.

Table 2. Means and analysis of variance for four seed quality traits in 15 genotypes of chickpea.

Variety	Seed weight(mg)	Starch content	Amylose %age	Amylopectin %age
CM-72	214.2	40.96	31.28	68.82
F88-83C	293.8	37.36	32.24	67.56
CA188220 X 88C003	387.6	40.24	36.2	63.8
CA188380 X 88C003	357.4	40.46	34.1	65.9
ILC -72	319.4	44.44	35.42	66.38
ILC-200	218.4	42.56	33.24	66.46
ILC-6090	304.8	39.94	33.94	66.06
FLIP84-86C	336.2	40.3	38.12	61.82
FLIP84-124C	291.2	39.66	42.54	59.46
FLIP85-58/SURUTATO-77	406.2	40.96	34.96	67.04
FLIP85-58/S.White	370.4	41.74	34.2	65.8
FLIP85-58C/S.White	416.2	42.24	37.58	64.42
FLIP 90-95C	328.4	42.22	37.44	62.56
FLIP 91-24	436.8	44.84	35.86	65.94
FLIP 91-62C	319.4	44.44	35.42	66.38
MS(VARIETIES)	21274.11	18.378	39.118	29.736
MS(REPLICATES)	657.6	3.488	13.741	4.00
MS(ERROR)	2064.589	2.494	6.737	6.365
F.RATI(V)	10.304**	7.368**	5.806**	4.672**
CD1	57.475	1.998	3.283	3.191
CD2	76.441	2.657	4.367	4.244

Although the absolute lavel of starch and amylose content in seed increases with increase in its size, the present investigation revealed intragenotypic differences for starch (% of seed dry weight) and amylose (% of starch) contents to be very small despite large differences for seed size. These differences were consistently similar in all the accessions. This type of persistent variation may be attributed to experimental error which was further supported from the fact that despite big differences for seed size within a genotype, the differences in starch and amylose contents were less. Moreover, there was no trend in variation which could be attributed to seed size or any other factor.

Of the 65% carbohydrate in chickpea 50% is in the form of starch (Fernandez & Berry, 1988). Starch and protein are the two major storage products in chickpea seed. The total amount of these storage products deposited during seed development predominantly determines the final size of the seed. Previous reports show that the contribution of starch to the dry weight of seed may range from 41-51% whereas the amylose percentage of starch have been reported to vary from 20-30% (Singh, 1985).

The range of variation for starch (38-45%) and amylose (32-43%) are generally not similar to those reported earlier. The differences between the results of two studies may partly be attributed to different genotypes studied in different environments and partly to analytical techniques which were different in both cases. Singh (1985) showed that amylose percentage of starch may be influenced by the environment Although the level of starch observed in genotypes used in the present study was generally different from those reported previously (Jambunathan & Singh, 1980). A difference of 5-7% for starch and 8-10% for amylose, observed between extreme genotypes of present study were similar to those recorded previously.

Report of Singh (1985) and the present results show that the general level of starch and amylose was similar to that of round pea (*RR* pea). Although considerable variation is present for starch and amylose content of starch in chickpea, there is no evidence of an equivalent effect to that found in pea induced by alleles at the *r* locus. Such a mutant would have total starch reduced from 50% to about 30% and the amylose content would be increased from about 35% to 70% (Koistra, 1962).

The genotypes used in the present investigation can not be exploited for genetic studies because of their small differences for starch and amylose. A wider screening of genotypes representing more geographical areas and seed size should therefore be done to find out lines with more differences. These lines could then be utilized for inheritance studies of starch and amylose.

The assessment of correlation between various quality traits of chickpea seed (seed size, starch content, amylose %age and amylopectin %age) revealed that there was no correlation coefficient greater than 0.5 both at genotypic and phenotypic levels. This indicates the lack of association between any of these quality parameters. However, previous reports show the possibility of association of high amylose and low starch in maize endosperm. Wang & Hedley (1991) reported that high amylose and low starch may not always be associated. In the present study, although there was no indication of significant association between any parameters, relatively high genotypic correlation coefficients between seed size, starch and amylose compared with that of phenotypic correlations gives clue of the trend of association at genotypic level.

Table 3. Genotypic (rG) and Phenotypic (rP) correlation coefficients between seed size (mg), Starch content (% of seed dry weight), Amylose (% of starch) and Amylopectin (% of starch) in 15 genotypes of chickpea.

		Seed size	Starch	Amylose	Amylopectin
Seed size		1			
Starch content	(rG)	.3129	1		
	(rP)	.1407			
Amylose content	(rG)	.3793	.0423	1	
	(rP)	.1332	.0521		
Amylopectin	(rG)	-.2346	.1215	-1.0466 1	
	(rP)	-.014	.1552	-.7502 **	

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