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CHANGES IN CARBOHYDRATES, AMINO ACIDS AND PROTEINS IN DEVELOPING SEED OF CHICKPEA*

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Key Word Index—*Cicer arietinum*; Leguminosae; chickpea; carbohydrates; proteins; amino acids; development.

Abstract—Developing seeds of chickpea cultivars G-130, L-550 and 850-3/27 grown under field conditions were sampled at different stages of maturity and analysed for soluble sugars, starch, soluble nitrogen, protein nitrogen and amino acids. Fr. wt of seeds of all three cultivars decreased after 28 days of flowering while the dry wt continued to increase. Rapid starch accumulation was observed between 14 and 28 days after flowering. Starch as per cent of seed dry wt started to decrease after 28 days, while starch per seed increased till maturity. The percentage of salt-soluble proteins decreased with maturation of seed. The electrophoretic pattern revealed that deposition of seed storage protein in cotyledons occurred 14 days after flowering. Most of the biochemical activity apparently occurred between 14 and 28 days after flowering.

INTRODUCTION

The role of grain legumes in human nutrition is well documented. Chickpea is one of the important grain legumes, ranking fifth in the total grain legumes production in the world. In India it accounts for *ca* 80% of the total production of all pulse crops. Protein and carbohydrates constitute *ca* 75% of the dry wt of mature chickpea seed [1].

The seed storage proteins of *Vicia faba* and *Pisum sativum* during development have been extensively studied [2–6]. The accumulation of carbohydrates and proteins during maturation of seeds in soybean [7], pigeonpea [8] and common beans [9] has been investigated and the composition of chickpea seed at different stages of maturation has also been studied [10, 11]. In the present work, we examined the changes in the levels of soluble sugars, starch, amino acids, soluble N and protein N with respect to seed dry matter accumulation throughout development. Using an electrophoretic technique, the seed storage proteins at different stages of maturation were also examined.

RESULTS AND DISCUSSION

Fr. wt and dry wt changes obtained from samples at different stages are shown in Fig. 1. The fr. wt increased up to 28 days after flowering and then a sharp decline was observed in all the cultivars (cvs). Rapid accumulation of grain matter was observed between 14 and 28 days after flowering. However, an indication of initiation of seed maturation was shown by a decline in the fr. wt of seeds. This maturation phase appeared to start 28 days after flowering (Fig. 1). Dry wt of the seed increased up to 35 days after flowering; thereafter only a very slight increase was recorded in all the cvs. The cv 850-3/27 attained the maximum seed wt.

Soluble sugars and starch content

The soluble sugars expressed as per cent of seed dry wt and as mg per dehusked seed are shown in Fig. 2. The percentage of soluble sugars continuously decreased up to 28 days after flowering and then remained unchanged till the grain matured. The amount of soluble sugars per dehusked seed, however, increased up to 35 days after

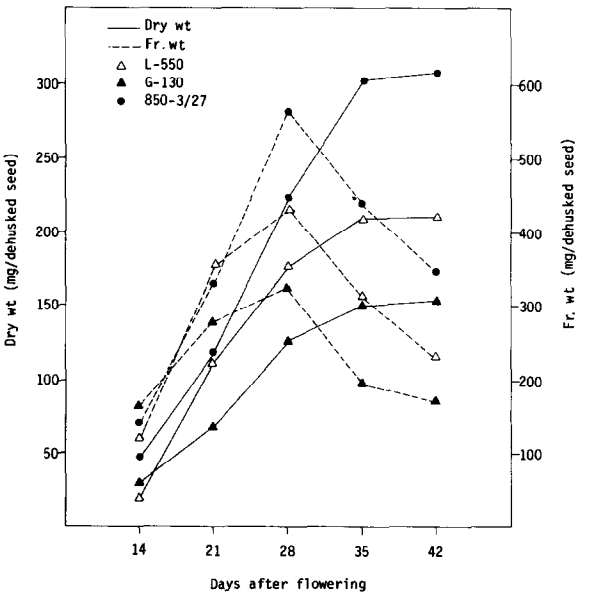

Fig. 1. Grain matter accumulation at different stages of maturation.

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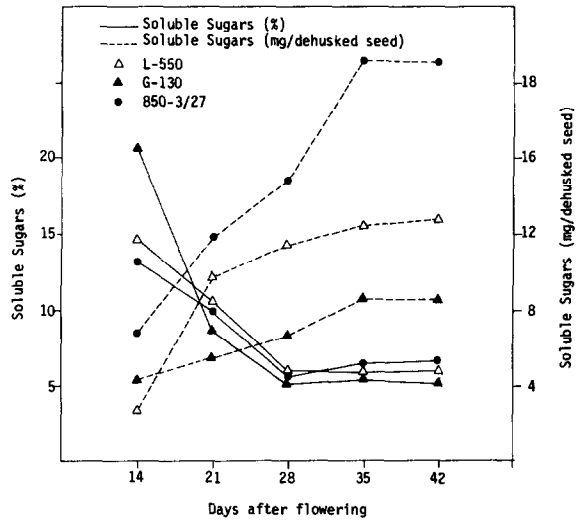

Fig. 2. Soluble sugars at different stages of maturation.

flowering and then stabilized. This change may be related to the increase in dry wt up to 35 days after flowering.

Changes in the levels of starch expressed as percentage of seed dry wt and amount per dehusked seed are shown in Fig. 3. On a percentage basis, a rapid increase was observed between 14 and 21 days after flowering. After reaching a maximum level at 28 days after flowering, the amount of starch in L-550 and G-130 decreased between 28 and 35 days after flowering, but it remained constant in 850-3/27. When the starch content, however, was expressed as mg per dehusked seed, it increased up to 35 days after flowering in all the cvs. Most of the starch accumulated during 14 to 28 days after flowering. The accumulation was more pronounced in 850-3/27. Since the accumulation of starch was accompanied by the changes in the amounts of soluble sugars during the same period, it appears that the period between 14 and 28 days after flowering is the period of intense biochemical activity. This observation is further supported by the findings discussed in the following section.

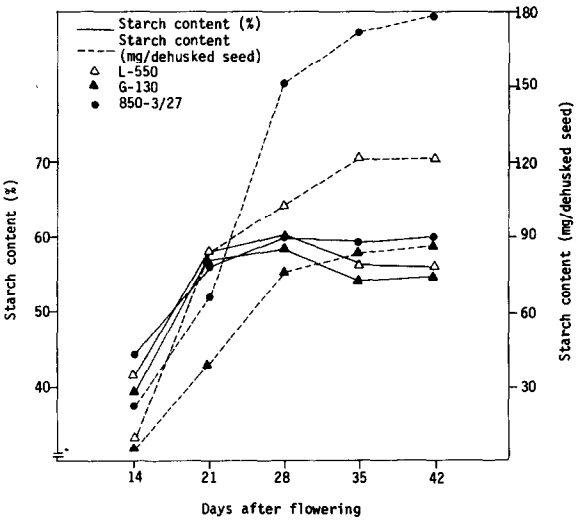

Fig. 3. Starch content at different stages of maturation.

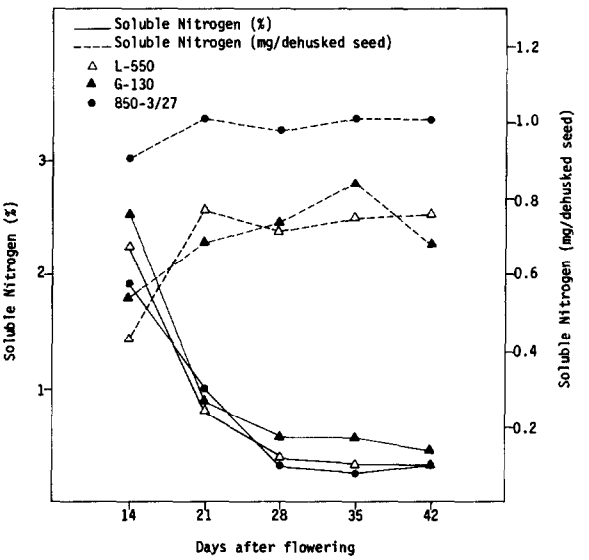

Fig. 4. Soluble nitrogen at different stages of maturation.

Soluble nitrogen and protein nitrogen

The concentration of soluble nitrogen expressed as per cent of seed dry wt and as mg per seed is shown in Fig. 4. Like soluble sugars, the percentage of soluble N also decreased up to 28 days after flowering and then remained constant till maturity. It is possible that during the early stages of development, soluble N was rapidly utilized for synthesis of protein which consequently increased. When the soluble N was expressed as mg per seed, it increased up to 21 days after flowering. After that there was no definite pattern in the case of L-550 and 850-3/27, though the latter showed the highest amount of soluble N per seed. In G-130 levels increased up to 35 days after flowering and then declined.

The results of accumulation of protein N expressed as per cent of seed dry wt and as mg per seed are shown in Fig. 5. Percentage of protein N increased slowly throughout the developmental stages in G-130 and L-550, but in 850-3/27 it increased up to 28 days after flowering and then

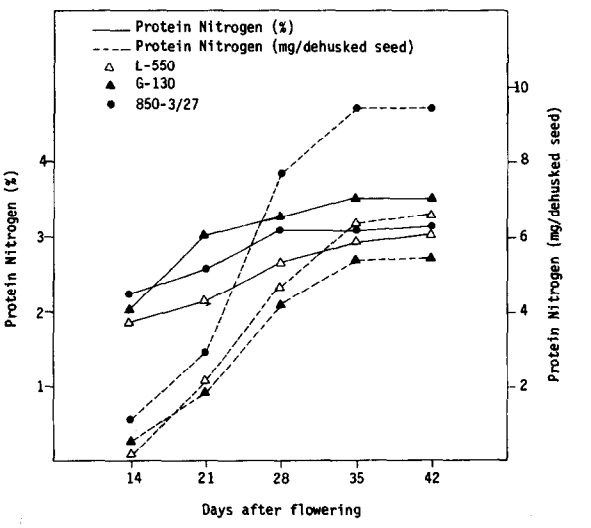

Fig. 5. Protein nitrogen at different stages of maturation.

Table 1. Level of salt-soluble proteins at different stages of maturation

	Days after flowering									
	14		21		28		35		42	
Cultivar	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
G-130	80.5	1.9	74.8	9.8	65.7	19.5	66.9	26.4	65.0	26.6
850-3/27	79.5	5.6	70.5	13.0	64.8	31.3	64.0	37.8	64.2	37.8
L-550	82.8	2.2	77.3	9.3	68.1	18.2	68.5	23.4	67.4	23.1

(a) Protein in the extracts was determined as described in ref. [19], and expressed as per cent of protein (N × 6.25) of the sample.
(b) mg salt-soluble protein/dehusked seed.

remained constant till maturity. The results of protein N expressed as mg per dehusked seed revealed that rapid protein deposition occurred during the period between 14 and 28 days after flowering, and again 850-3/27 exhibited the highest protein N per seed. However, unlike the percentage of protein, protein N per seed continued to increase up to 35 days after flowering in all the three cvs. The percentage of total N has been reported to decrease during maturation in chickpea [10]. We also observed that total N as per cent of dehusked dry seed decreased during maturation. We attribute this to the declining trend of soluble N because the percentage of protein N increased slowly throughout the developmental stages in G-130 and L-550, but in 850-3/27 it increased up to 28 days after flowering and then became constant.

Salt-soluble proteins and their electrophoresis

The salt-soluble proteins, albumins and globulins were determined for all the samples during the course of development and the results, expressed as per cent of protein N and as mg per dehusked seed, are shown in Table 1. The percentage of salt-soluble proteins decreased rapidly in the initial stages and after 28 days showed slight changes with the maturation of the grain. This implies that other proteins like glutelin and prolamine accumulate in higher proportion at later stages of maturation. But the amount of salt-soluble protein per seed also increased and this might be expected because of the increase in wt of dry seed.

In an attempt to observe the changes in the protein subunits during the developmental stages, electrophoresis of

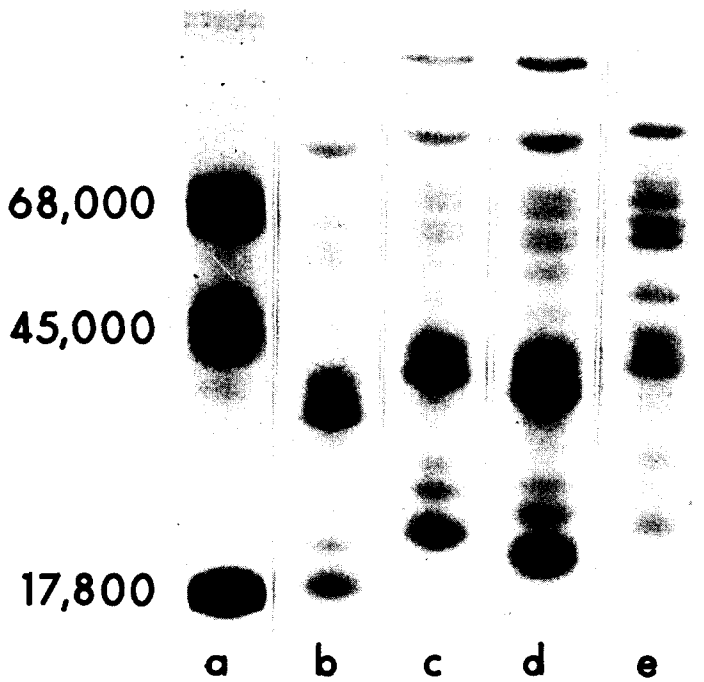

Fig. 6. Electrophoretic patterns of salt-soluble proteins of G-130: a, std proteins (human serum albumin 68 000, ovalbumin 45 000 and myoglobin 17 800); b, c, d and e samples of 21, 28, 35 and 42 days after flowering, respectively.

salt-soluble proteins was carried out on samples that had equal concentration of protein. Protein bands were absent in samples obtained 14 days after flowering. It appears, therefore, that only small polypeptides accumulated up to 14 days after flowering. In the case of samples at 21 days after flowering, the protein bands of varying intensity appeared in both the cvs studied (Figs. 6 and 7). As the major protein fraction in chickpea is legumin, the heavily stained protein sub-units of MW *ca* 40000 and 20000 are probably due to this protein. Earlier workers [12] have reported similar observations on chickpea and the observed small variation in the mobilities of these bands reported here may be due to the different running conditions. This indicates that storage protein synthesis was initiated during the period between 14 and 21 days after flowering. Earlier workers also reported that synthesis of seed storage proteins, vicilin and legumin started after 10 days of flowering in *Pisum sativum* [5], and after 14 days of flowering in *Cajanus cajan* [8].

Amino acid composition of proteins

The amino acid composition of seed proteins of G-130 and L-550 is presented in Tables 2 and 3, respectively. Cystine was not detected in the samples obtained at later stages of maturation and the reported values at early stages of maturation are probably low. This may be attributed to the partial destruction of this amino acid as 0.1 M NaOH was used in our procedure for dissolving the residue after removal of soluble N. Earlier workers [13, 14] reported that alkaline extraction of soybean meal resulted in the destruction of cystine and the formation of lysinoalanine.

The concentrations of various amino acids changed considerably during the course of development. Histidine and arginine increased with maturation of grain. Glutamic acid increased up to 42 days after flowering in L-550, whereas in G-130 it increased up to 21 days after flowering and became more or less constant during the later stages. Sulphur-containing amino acids, methionine and cystine decreased as the grain matured. Valine also declined during the course of development. Lysine showed a slight increase up to 21 days after flowering and was relatively constant afterwards. No definite trend was observed with respect to the composition of other amino acids during the course of development. Earlier workers [11] reported the amino acid composition of total crude protein of the seed, while our report pertains to amino acid composition of seed protein obtained after TCA precipitation.

EXPERIMENTAL

Chickpea cvs G-130, L-550 and 850-3/27, which are widely cultivated in India, were used in the present study. The crops were grown during 1977–1978 under normal cultural practices in the experimental plots of the Pulse Physiology program at the ICRISAT Centre, near Hyderabad, India. The plants that flowered on the same day were chosen at random and the flowers were tagged. The pod samples at 14, 21, 28, 35 and 42 days after flowering were collected and chilled in ice. Seeds were removed from the pods and subsequently seed coats were removed manually. Samples were then freeze-dried and ground to pass through a 100-mesh sieve. Samples at 7 days after flowering were not taken for this study because of their very small size.

Total N *and protein* N. In this study, protein N is defined as the difference between the total N and soluble N. For the extraction

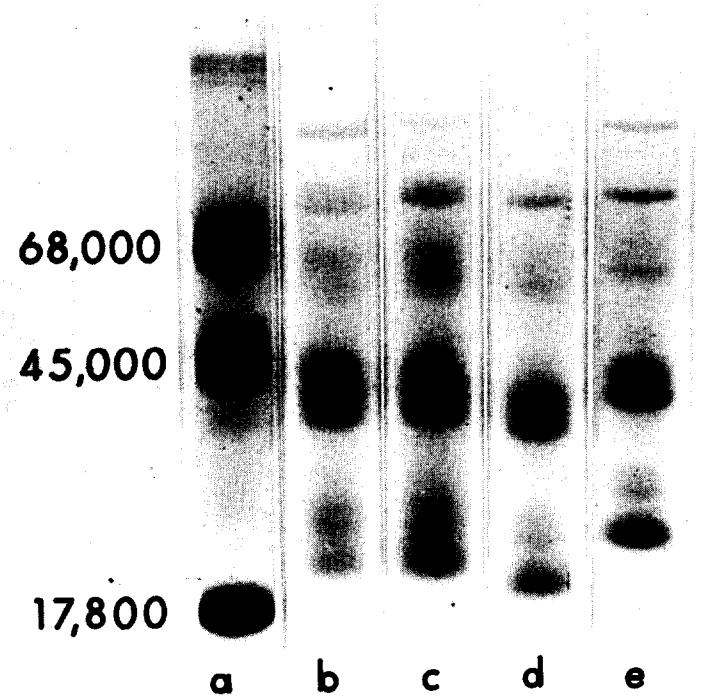

Fig. 7. Electrophoretic patterns of salt-soluble proteins of L-550: a, std proteins (human serum albumin 68 000, ovalbumin 45 000 and myoglobin 17 800); b, c, d and e samples of 21, 28, 35 and 42 days after flowering, respectively.

Table 2. Amino acid composition of seed proteins of G-130 at different stages of maturation*

Amino acid	Days after flowering				
	14	21	28	35	42
	(g/16 g N)				
Lysine	7.2	8.0	7.9	7.2	7.1
Histidine	0.6	0.6	1.8	1.6	2.1
Arginine	3.6	4.6	5.4	5.9	6.4
Aspartic acid	11.6	13.7	12.8	13.4	13.6
Threonine	4.1	3.9	3.4	3.7	4.1
Serine	4.3	5.3	4.4	4.6	4.6
Glutamic acid	16.0	20.8	20.1	20.6	20.4
Proline	5.0	5.1	4.6	4.8	4.9
Glycine	5.4	5.1	4.0	5.0	5.2
Alanine	5.7	5.5	4.7	4.9	5.1
Valine	7.4	6.1	6.0	5.5	5.4
Cystine	0.3	0.2	0.1	0.1	—
Methionine	1.6	1.2	1.2	1.0	1.0
Isoleucine	4.4	4.9	4.4	4.7	4.4
Leucine	9.8	10.6	8.6	9.0	9.8
Tyrosine	2.1	1.3	2.7	3.2	3.0
Phenylalanine	6.5	7.5	6.1	6.8	6.9
Total	95.6	104.7	98.1	102.0	91.7
Nitrogen recovery (%)	87.2	93.4	90.6	92.5	84.0

* Based on protein nitrogen.

Table 3. Amino acid composition of seed proteins of L-550 at different stages of maturation*

Amino acid	Days after flowering				
	14	21	28	35	42
	(g/16 g N)				
Lysine	7.3	8.3	7.9	7.5	7.6
Histidine	0.9	0.9	1.2	1.8	2.4
Arginine	6.4	6.4	6.7	6.7	7.5
Aspartic acid	12.7	12.9	12.6	12.7	13.5
Threonine	4.4	3.5	1.4	3.1	3.4
Serine	4.8	4.7	3.4	4.5	4.9
Glutamic acid	16.4	18.9	18.4	19.5	20.4
Proline	4.9	4.4	4.5	4.2	4.6
Glycine	5.2	4.5	4.9	5.0	4.4
Alanine	6.6	5.3	4.9	3.4	4.7
Valine	6.4	6.0	5.5	4.6	4.5
Cystine	0.4	0.2	0.2	—	—
Methionine	1.9	1.1	1.2	1.1	1.1
Isoleucine	5.2	4.8	4.6	4.2	4.1
Leucine	9.2	9.7	9.7	8.5	8.8
Tyrosine	1.7	1.4	1.9	2.6	2.8
Phenylalanine	5.9	5.8	5.9	5.4	5.5
Total	100.3	98.8	90.0	94.8	100.7
Nitrogen recovery (%)	92.7	92.5	83.9	86.4	90.3

* Based on protein nitrogen.

of soluble N, different concns of TCA were studied and N solubility was observed to be minimal at 10% TCA concentration. Further, using the biuret procedure it was observed that 10% of TCA solubilized very negligible amount of protein [15]. Therefore, soluble N was determined using 10% TCA according to the procedure reported earlier [8]. Standard micro-Kjeldahl method [16] was used for the determination of soluble N in the aliquots and total N on freeze-dried and defatted samples. The coefficients of variation for the estimation of total N and soluble N were 1.39 and 1.60%, respectively.

Soluble sugars and starch content. Freeze-dried and defatted samples were used for the extraction of soluble sugars using 80% EtOH in a Soxhlet apparatus and determination by the PhOH–H_2SO_4 method [17]. Starch content in the residue was determined by the procedure of enzymatic hydrolysis [18] using glucoamylase as described earlier [8]. Mean coefficient of variability for soluble sugars estimation was 2.04%.

Amino acid composition. The residue obtained after removing the soluble N was dissolved in 0.1 M NaOH and an aliquot containing *ca* 1.5 mg N was taken into a round-bottom flask and neutralized by addition of 0.1 M HCl. HCl (11 M) was then added to bring the final concn to 6 M HCl and the contents refluxed for 24 hr. After evaporating the HCl from the hydrolysate, the residue was dissolved in citrate buffer (pH 2.2). The amino acids were analysed in a Beckman 120C amino acid analyser. Tryptophan was not analysed. Limits of reproducibility varied between 2 and 10%.

Extraction and electrophoresis of salt-soluble proteins. The defatted samples (500 mg) were extracted with 10 ml 0.5 M NaCl in 0.01 M Pi buffer (pH 7) by shaking in a centrifuge tube for 1 hr and the extracts were centrifuged at 10000 *g* for 15 min. The residue was reextracted twice by shaking for 30 min each time and the supernatants were pooled and made to 50 ml in a flask. Protein content in the aliquots was estimated by the procedure described in ref. [19]. SDS-acrylamide gel electrophoresis was performed as described in ref. [20]. Protein samples were treated with 0.1% SDS and 0.1% 2-mercaptoethanol in 0.01 M Pi buffer, pH 7.1, for 2 min at 100°. An aliquot containing 200 mg of protein was applied on each of the gel. Human serum albumin (68000), ovalbumin (45000) and myoglobin (17800) were used as marker proteins.

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