Phytochemistry, 1980, Vol. 19, pp. 1291–1295. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0701-1291 $02.00/0

BIOCHEMICAL CHANGES IN DEVELOPING SEEDS OF PIGEONPEA (*CAJANUS CAJAN*)*

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(*Revised received* 15 *October* 1979)

Key Word Index—*Cajanus cajan*; Leguminosae, pigeonpea; carbohydrates; proteins; amino acids; development.

Abstract—Soluble sugars, starch, soluble nitrogen and protein nitrogen were studied in developing seeds of 3 cultivars of pigeonpea. When expressed on a per seed basis soluble sugars increased up to 35 days after flowering and then declined slightly. Rapid starch accumulation was observed between 14 and 28 days after flowering. The levels of soluble nitrogen and protein nitrogen underwent rapid changes during the same period. Amino-acid composition of seed protein was also studied at different stages of maturation. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of salt-soluble proteins revealed that seed storage globulins are formed after 14 days of flowering and do not change much during later stages of maturation.

INTRODUCTION

Carbohydrates and proteins are the main constituents of many legume seeds. During the maturation of legume seeds, these constituents are stored in the cells of cotyledons.

Pigeonpea is one of the important pulse crops in India and is grown in the tropical and subtropical regions of the world. Its seeds are an important source of protein in the human diet. Changes in the protein nitrogen and soluble nitrogen of developing and germinating seeds of pigeonpea have been reported recently [1]. So far as the authors are aware, information is not available on the changes in the carbohydrates, amino acids and proteins in developing seeds of pigeonpea. In this paper we report the results of such a study.

RESULTS AND DISCUSSION

Changes in dry and fr. wt observed for 3 cultivars (cvs) of pigeonpea are shown in Fig. 1. Between 14 and 28 days after flowering, a large increase in seed size was observed. Fr. wt per seed reached a maximum 28 days after flowering, whereas dry wt per seed increased up to 35 days after flowering in all the cvs studied. After 35 days of flowering, a slight increase in dry seed wt was observed in the case of Hy-3c and ICP-1 and did not change much in the case of ST-1.

Soluble sugars and starch content

Changes in the level of soluble sugars during maturation are shown in Fig. 2. Soluble sugars, as per cent of the dry wt, continously increased up to 14 days after flowering, then showed a sharp decline and thereafter remained more or less unchanged. However, when the soluble sugars were expressed as mg per dehusked seed, an increasing trend was observed up to 35 days after flowering and then declined slightly.

Starch content in the developing grains was determined and changes in starch level as per cent of dry wt and mg per dehusked seed are plotted in Fig. 3. Dry wt of starch content at 14 days after flowering was 17.4% in ST-1, 20% in Hy-3c, and 27% in ICP-1 and this increased to 61.9, 63.2 and 62.4%, respectively, 21 days after flowering. But the amount of starch per dehusked seed continued to increase up to 35 days after flowering in all cvs studied and then it showed slight changes towards maturation. It appeared that rapid starch accumulation occurred during the period between 14 and 28 days after flowering. This was also accompanied by changes in the levels of soluble sugars during the same period. Hence, there appeared to be an intense biochemical activity during the period between 14 and 28 days after flowering.

Soluble nitrogen and protein nitrogen

The percentage of soluble nitrogen and soluble nitrogen per seed in these cvs are plotted in Fig. 4. The soluble nitrogen as per cent of dry wt increased up to 14 days after flowering, then showed a sharp decline and became constant at later stages of maturation. On the other hand, soluble nitrogen expressed as μg per seed increased up to 28 days after flowering, and then became constant in the case of ICP-1 but showed a slight increase in the case of Hy-3c and ST-1. Soluble

* Contribution from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Published with the approval of the Director as Journal Article No. 102.

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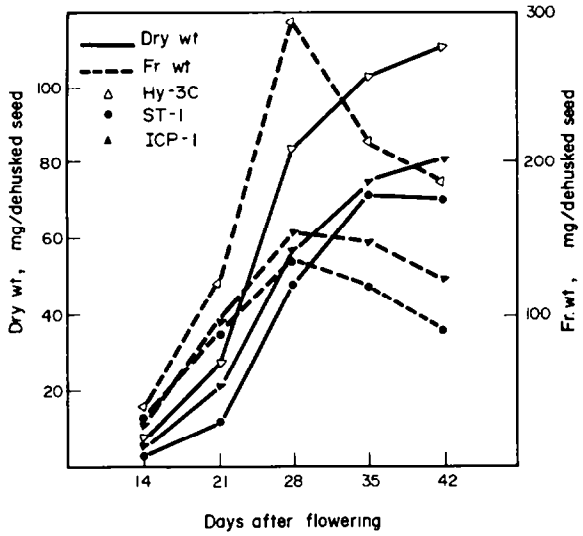

Fig. 1. Grain matter accumulation at different stages of maturation.

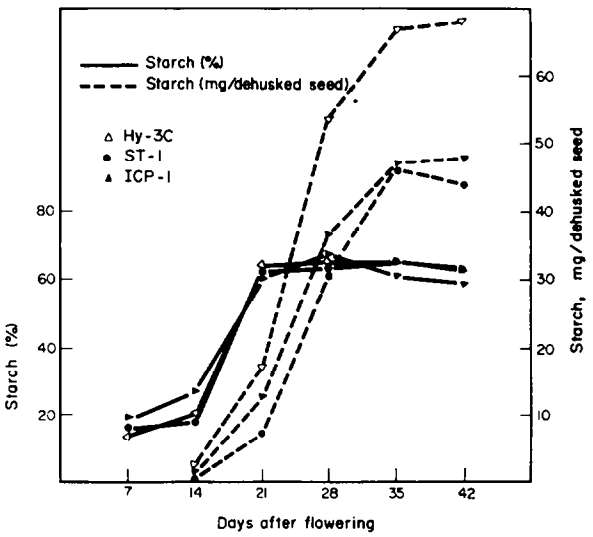

Fig. 3. Starch content at different stages of maturation.

nitrogen calculated as proportion of the protein nitrogen (values not reported) increased up to 14 days after flowering and then progressively decreased as the grain developed. However, a sharp decline in the levels of soluble nitrogen during the period between 14 and 28 days after flowering (Fig. 4) indicated that during this period the amino acids were utilized at a higher rate for protein synthesis. Furthermore, this observation was confirmed by electrophoresis as seed storage proteins could be detected after only 14 days of flowering.

Protein nitrogen values in these cvs were obtained by subtracting soluble nitrogen from total nitrogen. Protein nitrogen, expressed as per cent of dry wt, decreased gradually up to 21 days after flowering in all the 3 cvs, and then remained more or less unchanged except for the ST-1 which showed a slight increase 35 days after flowering (Fig. 5). The amount of protein, expressed as mg per seed, increased up to 35 days after flowering and thereafter declined in the case of ST-1 and slightly increased in the case of Hy-3c and ICP-1.

Electrophoretic patterns of salt-soluble proteins

Extracts of salt-soluble proteins of Hy-3c and ST-1 were studied for their electrophoretic patterns. Protein bands were not detected in the samples taken 7 and 14 days after flowering, although Lowry's protein reaction was positive. This could be attributed to the presence of small peptides in the early stages of maturation. The protein bands of varying intensity appeared at 21 days after flowering. The salt-soluble proteins had several sub-units that appeared in the order of decreasing MW (Figs. 6 and 7). The seed globulins of mature pigeonpea seed, consisting of 9 sub-units, have been reported by others [2]. However, it would be difficult to explain the changes that occur

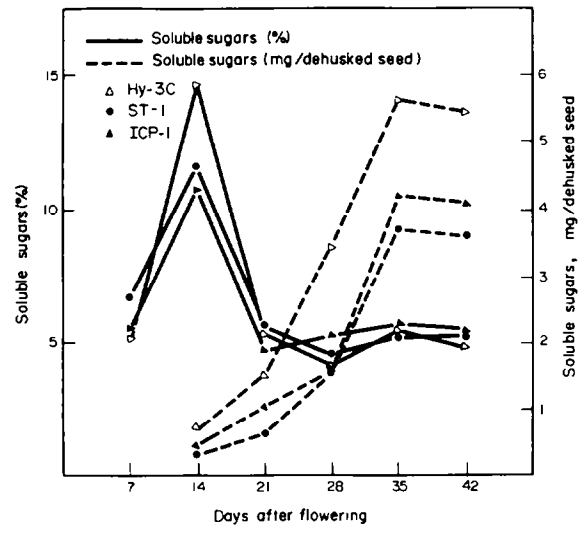

Fig. 2. Soluble sugars at different stages of maturation.

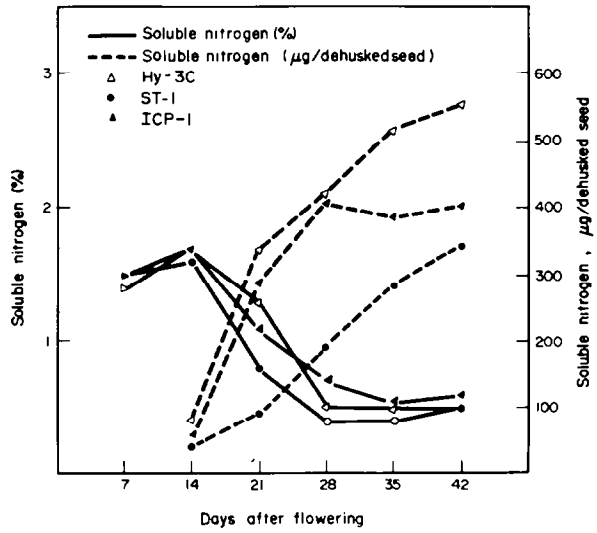

Fig. 4. Soluble nitrogen at different stages of maturation.

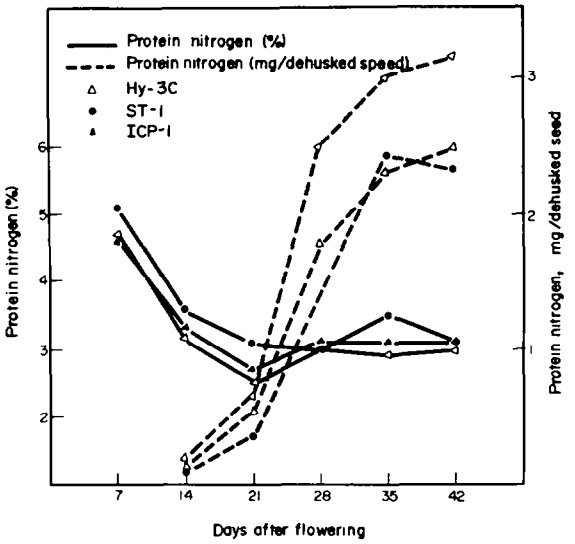

Fig. 5. Protein nitrogen at different stages of maturation.

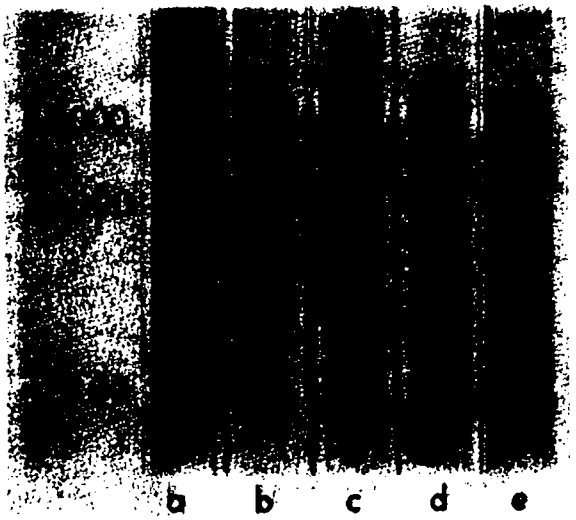

Fig. 6. Electrophoretic patterns of seed storage proteins during maturation of Hy-3c. a: Std proteins; b, c, d and e: samples from 21, 28, 35 and 42 days after flowering, respectively.

with respect to different globulins which may have similar electrophoretic mobilities [3]. Several investigators have employed electrophoretic techniques to study the changes in the proteins at different stages of maturation [4, 5]. As shown in Figs. 6 and 7, high MW protein components appeared to be the major seed proteins of pigeonpea in samples obtained 21 days after flowering. As we could not detect any bands in the samples taken 7 and 14 days after flowering, it appears that the protein synthesis starts between 14 and 21 days after flowering. Earlier workers [6] have also reported that protein synthesis in the case of *Pisum sativum* begins 8 days after flowering.

Amino acid composition of proteins

Protein amino acids of Hy-3c and ST-1 at different stages of maturation were determined (Tables 1 and

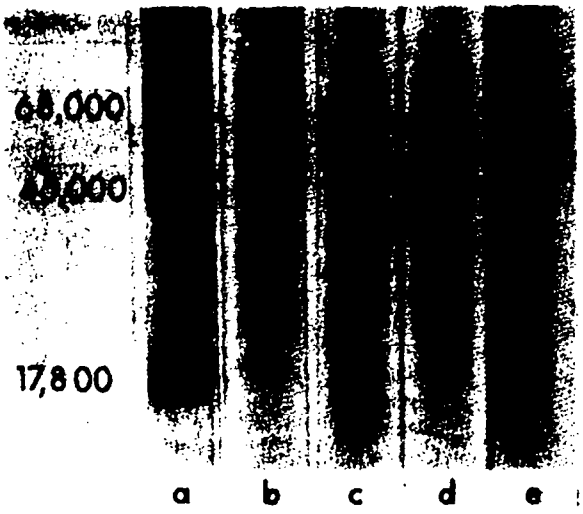

Fig. 7. Electrophoretic patterns of seed storage proteins during maturation of ST-1. a: Std proteins; b, c, d and e: samples from 21, 28, 35 and 42 days after flowering, respectively.

2). Tryptophan was not analysed. Cystine was not detected in the few samples obtained from the last stages of maturation, and the values are probably low for the samples of early stages of maturation. The reason for this is the partial destruction of this amino acid when 0.1 N NaOH was used in our procedure for dissolving the residue after removal of soluble nitrogen. Earlier workers [7, 8] have reported that alkaline extraction of soybean meal resulted in the destruction of cystine and the formation of lysinoalanine.

Tables 1 and 2 show that the levels of many essential and non-essential amino acids changed significantly in both cvs, although no remarkable differences among them were revealed. The limiting essential amino acids, methionine and cystine, decreased during grain maturation. Leucine, isoleucine, valine and glycine decreased, whereas phenylalanine variably increased during grain maturation. Glutamic acid in Hy-3c gradually increased up to 35 days of flowering and up to 28 days in ST-1; it decreased at later stages of maturation. No definite trend was observed for other amino acids.

The limiting essential amino acids, methionine and cystine, are lower in the protein of mature cotyledons. The lower content of these amino acids might be due to (1) increase in storage proteins, which are relatively low in sulphur amino acids at later stages of development; (2) selective degradation of certain proteins during maturation and (3) lack of synthesis of some specific proteins because of lack of these amino acids in the free pool.

EXPERIMENTAL

Pigeonpea cvs, Hy-3c, ST-1 and ICP-1, grown at ICRISAT Centre near Hyderabad, India during 1976–1977 were tagged at flowering, and samples were collected periodically from 7 days after flowering to maturity. Seeds were removed from the pods, chilled in ice, and the seed coats were removed manually. Samples were then freeze-dried and ground to 100-mesh powder. Samples collected at 7 days

Table 1. Amino acid composition of seed proteins of Hy-3c at different stages of maturation

	Days after flowering					
	7	14	21	28	35	42
	(g/16 g N*)					
Lysine	7.5	6.4	7.5	7.3	7.4	7.2
Histidine	1.2	1.0	1.0	1.2	0.7	1.4
Arginine	4.4	4.1	4.4	4.1	3.9	4.1
Aspartic acid	12.3	11.8	12.1	12.3	11.8	11.2
Threonine	4.3	4.2	3.7	3.4	2.4	3.4
Serine	4.9	3.7	4.9	5.0	3.8	5.3
Glutamic acid	15.9	17.7	19.8	21.1	22.3	20.4
Proline	5.4	6.3	5.0	5.1	5.2	5.4
Glycine	5.9	5.4	4.9	4.4	4.0	4.3
Alanine	7.0	8.0	5.9	5.6	6.8	5.2
Cystine	0.3	0.3	0.2	0.2	—	—
Valine	7.5	7.4	6.8	5.6	6.2	5.4
Methionine	1.8	1.5	1.4	1.4	0.9	0.8
Isoleucine	4.9	5.0	4.2	4.1	4.3	4.0
Leucine	11.2	13.1	10.4	10.7	9.2	9.9
Tyrosine	3.2	3.3	2.6	1.8	1.2	2.1
Phenylalanine	5.6	6.2	7.1	7.9	6.9	8.0
Total	103.3	105.4	101.9	101.2	97.0	98.1
Nitrogen recovery (%)	91.4	93.8	92.6	86.2	88.5	85.6

* Based on protein nitrogen.

Table 2. Amino acid composition of seed proteins of ST-1 at different stages of maturation

	Days after flowering					
	7	14	21	28	35	42
	(g/16 g N*)					
Lysine	7.2	6.2	7.3	7.4	7.5	7.4
Histidine	1.1	1.4	0.7	1.2	2.4	2.7
Arginine	4.4	3.8	4.2	3.9	4.1	4.0
Aspartic acid	12.7	12.2	12.9	11.9	11.1	11.3
Threonine	4.4	4.3	3.8	3.3	3.4	3.2
Serine	4.9	4.7	5.2	4.8	4.7	4.6
Glutamic acid	15.5	16.4	19.9	21.7	19.7	19.3
Proline	5.6	5.3	5.2	5.0	4.9	4.6
Glycine	6.2	5.7	4.9	4.3	4.4	4.2
Alanine	7.1	6.7	5.7	5.0	5.1	5.1
Cystine	0.4	0.4	0.2	—	0.1	0.1
Valine	7.7	7.5	6.6	5.4	5.2	5.1
Methionine	1.8	1.6	1.2	1.0	1.2	0.9
Isoleucine	5.1	5.0	4.4	3.9	3.7	3.6
Leucine	11.2	10.8	10.8	9.5	9.1	8.8
Tyrosine	3.8	4.0	2.4	2.7	2.7	3.1
Phenylalanine	6.0	6.3	7.8	7.6	7.6	7.2
Total	105.1	102.3	103.2	98.5	96.9	95.2
Nitrogen recovery (%)	96.8	94.2	94.5	90.3	87.6	84.7

* Based on protein nitrogen.

were left intact due to their small size and the difficulties of removing the seed coats.

Total nitrogen and protein nitrogen. The standard micro-Kjeldahl method [9] was used for the determination of soluble and total nitrogen in freeze-dried and defatted samples. For the extraction of soluble nitrogen different concns of TCA were studied, and at 10% TCA, nitrogen solubility was minimal. Further, using the biuret procedure it was observed that 10% TCA solubilizes a negligible amount of protein. Therefore, the soluble nitrogen was determined as follows. Freeze-dried and defatted samples (150 mg) were homogenized with 15 ml 10% (w/v) TCA and then shaken for 30 min in a mechanical shaker. The material was centrifuged (12 000g for 15 min) and the residue washed twice with 10% TCA. The supernatant and the washings were pooled and made up to 25 ml. Aliquots were used for N

estimation by the micro-Kjeldahl method. Protein N was calculated by subtracting the levels of soluble N from the total N.

Soluble sugars and starch content. Sugars were extracted from the freeze-dried and defatted sample, using 80% EtOH in a Soxhlet apparatus. Extracts were evapd to dryness and the residue taken up in H_2O. Aliquots were used for estimation of soluble sugars by the PhOH–H_2SO_4 method [10]. Starch content in the residue was determined by enzymatic hydrolysis [11], with minor modification as described below. Dried residue (70 mg) was placed in a conical flask and a few drops of EtOH and 10 ml of H_2O were added. Contents were dispersed and then autoclaved for 90 min at 1.34 kg/cm^2 (125°). The suspension was cooled to room temp. and 1 ml 2 M acetate buffer (pH 4.8) was added and the final vol. was made to 25 ml. An aliquot was incubated with 25 mg of glucoamylase (Sigma) for 2 hr at 55°. The glucose thus released was estimated, using the PhOH–H_2SO_4 method.

Amino acid composition. The residue obtained after removing the soluble N was dissolved in 0.1 N NaOH and the N content in the aliquot was determined by the micro-Kjeldahl method. A suitable quantity of the aliquot (1.5–1.6 mg N) was taken and neutralized by the addition of 0.1 N HCl. Conc HCl was then added to bring the final conc to 6 N HCl and the contents were 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 120 C amino acid analyser. Mean coefficient of variability of analysis for different amino acids ranged between 1.3 and 9.0% except in the case of histidine and isoleucine where it was observed to be 11.9 and 11.3%, respectively.

Extraction and electrophoresis of salt-soluble proteins. Seed proteins were extracted with 0.5 M NaCl in 0.01 M Pi buffer (pH 7) by shaking in a centrifuge tube for 1 hr using a flour to solvent ratio of 1:20 (w/v). Extracts were clarified by centrifugation at 10 000 g for 15 min and used for electrophoresis. Protein content was estimated by the procedure of ref. [12]. SDS-acrylamide gel electrophoresis was performed according to the procedure of ref. [13] with minor procedural modifications in that the proteins 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 μg protein was applied on each of the gel. Electrophoresis was carried out using 7.5% acrylamide gels in Pi buffer (pH 7.1) for 3.5 hr at 5 mA/tube. Marker proteins used were human serum albumin (68 000), ovalbumin (45 000) and myoglobin (17 800). Gels were stained in 0.25% Coomassie Brilliant Blue-R 250 in MeOH–H_2O–HOAc (5:4:1). Destaining was accomplished in the same solvent with a ratio of 5:88:7.

Acknowledgements—The authors wish to thank Mrs. Santosh Gurtu and Mr. A. L. Reddy for technical assistance during the investigation.

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