

degree than samples simply kept frozen. Therefore, the lyophilized samples were used for the comparison. Crude extracts of the various samples were dissolved in D<sub>2</sub>O solutions containing a known concentration of TSP, and the integral of the isolated C-2 doublet signal (7.78 ppm) was compared to that of TSP in the <sup>1</sup>H NMR spectrum. This allowed for a determination of the total amount of ranunculin in a given crude extract, the results of which are reported in Table I. The growth stage with the highest concentration of ranunculin proved to be the "early flower" stage, accounting for 2.3% of the dry weight of the plant.

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## Amino Acid Composition of Developing Pigeon Pea (*Cajanus cajan*) Seeds

The amino acid composition of seed proteins and free amino acid pool of pigeon pea (*Cajanus cajan*) was investigated at different developmental stages after fertilization. The nitrogen content of pod covers increased up to 21 days followed by a gradual decline, while that of seeds increased sharply from 21 to 42 days. The free amino acid content of seeds was maximum between 21 and 28 days. The decrease in free amino acids after 28 days was accompanied by a rapid accumulation of protein up to 42 days in the developing seed. The amino acids cysteine, valine, isoleucine, and methionine were generally absent in the free pool after 28 days of seed development. The pigeon pea proteins were found to be very poor in methionine, tyrosine, and histidine.

Pigeon pea is an important source of dietary proteins in many developing countries, particularly in the semiarid regions. It is a well-recognized fact that to increase the productivity of legumes the foremost consideration is the stabilization of their yields by breeding techniques. However, to breed for their protein quality, it is necessary to understand the metabolic changes that occur in the seeds as they mature. Sulfur-containing amino acids and threonine are reported to be the most limiting amino acids in chickpea proteins (Kaul and Gassi, 1971). Since legumes are generally rich in the essential amino acid lysine, they play an important role in supplementation of cereal-rich diets (Chatterjee et al., 1977). In chickpea accumulation of protein fractions during development of cotyledons has been studied (Srivastava et al., 1981). However, there are no available data on the metabolic changes of developing pigeon pea seed. The present study was undertaken to ascertain the changes in the free amino acid pool, amino acid composition of seed proteins, protein content, and total nitrogen content of seeds and pod covers during their different stages of development.

#### MATERIALS AND METHODS

**Plant Material.** Pigeon pea (*Cajanus cajan*) cultivar "Sharda" was grown under field conditions on red soils at the ICRIAT farm at Patancheru. The flowers were

tagged on the day of their opening. The pods for analysis were collected at 7, 14, 21, 28, 35, 42, and 49 days after the opening of flowers from different plants. The grains matured at 49 days after flowering.

**Total Nitrogen.** Seeds were separated from the pods by hand dissection, and the fresh weight was taken of both seeds and pod covers. Seeds and pod covers were oven-dried to constant weight. Dried seeds and pod covers were ground by a cyclone mill, and the total nitrogen was determined in duplicate by the micro-Kjeldahl method (AOAC, 1970).

**Amino Acid Analysis and Protein Determination.** Freshly weighed and counted seeds, collected at different maturity stages, were heated for 30 min at 80 °C in 5% trichloroacetic acid (TCA). The samples were homogenized (0.2 g/mL) in TCA in a Potter-Elvehjem homogenizer for 3 min and centrifuged at 2000g at room temperature for 15 min. The supernatant was saved and extracted with ether to remove TCA. Finally, the samples were evaporated to dryness under vacuum and dissolved in 0.2 M citrate buffer, pH 2.88, for quantitative determination of free amino acids. The pellet was washed twice by suspending in fresh 5% TCA and recentrifugation. After being washed, the TCA precipitate was dissolved in 1 N NaOH. Protein hydrolysis was performed as described earlier (Yadav et al., 1972). Amino acids in TCA super-

Table I. Fresh Weight and Dry Weight of Developing Seeds and Pod Covers of Pigeon Pea<sup>b</sup>

days after opening of the flower	seeds		pod covers	
	fresh wt, mg/seed	dry wt, mg/seed	fresh wt, mg/PC <sup>a</sup>	dry wt, mg/PC
7	0.80	0.15	16.10	7.56
14	10.62	2.87	125.85	59.30
21	80.97	13.10	290.45	93.00
28	182.23	48.10	323.60	102.20
35	219.75	70.00	392.60	83.90
42	141.35	77.50	150.00	91.80
49	103.30	81.20	108.40	95.70

<sup>a</sup> PC = pod cover. <sup>b</sup> Values given in the table are an average of duplicate analyses.

nant and protein hydrolysates were determined by use of a Beckman amino acid analyzer, Model 120C. The amino acid recoveries varied from 85 to 88%. Cystine was not determined.

Protein content of the dissolved TCA precipitates was estimated by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

**Fresh Weight and Dry Weight.** Fresh and dry weights of developing pigeon pea pod covers and seeds are presented in Table I. The fresh weight of the pod covers and seeds increased until 35 days and then decreased gradually as the seed matured. The dry weight of the seeds increased throughout development. The maximum accumulation of 70% of the total dry weight occurred between 21 and 35 days. In contrast, the dry weight of pod covers increased up to 28 days followed by a decline. Seed maturation was initiated by the onset of desiccation as observed by the decrease in fresh weight of seeds and pod covers after the 35 days.

**Nitrogen and Protein Contents.** The total nitrogen content in seeds and pod covers and the protein content of seeds at different stages of maturation are summarized in Figure 1. The nitrogen content in pod covers increased sharply up to 21 days and declined gradually afterward. In contrast, the total nitrogen content of the developing seeds started to increase after 14 days of fertilization and continued to increase sharply up to 42 days followed by a slight decline.

On the other hand, protein accumulation was slower up to the first 21 days as only 14% of the total protein was

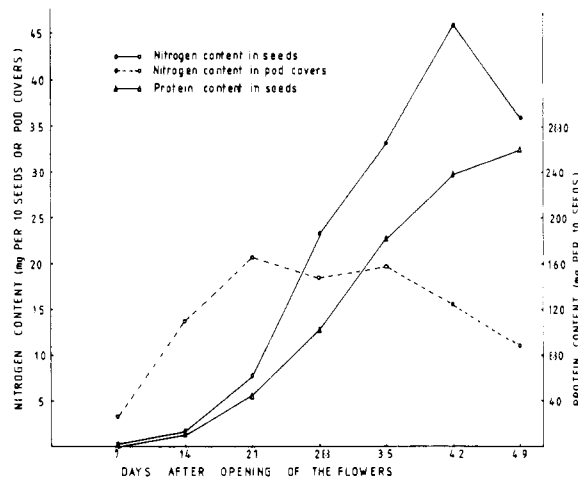


Figure 1. Changes in nitrogen and protein content. The total nitrogen content in seeds, total nitrogen content in pod covers, and protein content in seeds are expressed in milligrams per 10 seeds or pod covers on a dry matter basis. All the values are an average of two determinations.

deposited during this period, while in the subsequent same period of development, between 21 and 42 days, 75% of the total protein was deposited. From 42 days to maturity 8% of the total protein accumulated. The increase in protein content of seeds was accompanied by a simultaneous increase in the dry weight from 21 to 42 days. However, the decrease in the nitrogen content and dry weight of pod covers after 21 and 28 days, respectively, may have been due to translocation of photosynthetic and nitrogenous metabolites to seeds. The changes in nitrogen content of pigeon pea seeds are different from those observed in maturing cereal grains. In wheat there is an initial decrease in nitrogen content due to rapid starch synthesis during early stages of kernel development (Jennings and Morton, 1963; Dexter and Dronzek, 1975, 1976). These differences may thus reflect different rates of starch deposition at various stages of seed development in these crops. The decrease in nitrogen content of pod covers after 21 days might be due to translocation of nitrogenous precursors from pod covers to developing seeds.

**Free Amino Acid Pool.** The free amino acid composition of the developing pigeon pea seeds is presented in

Table II. Amino Acid Composition of the Free Pool of Amino Acids in Maturing Pigeon Pea Seeds (Millimoles per 10 Seeds)<sup>c</sup>

amino acid	days after opening of the flower						
	7	14	21	28	35	42	49
Lys	0.008	0.085	0.483	1.168	0.525	0.440	0.634
His	0.019	1.055	3.492	3.302	0.788	0.101	0.269
Arg	0.022	0.195	2.892	6.221	3.825	1.609	5.993
Asp <sup>a</sup>	0.051	1.141	4.196	1.378	1.381	2.316	n.f.
Thr	0.027	1.033	2.053	4.617	0.757	1.279	n.f.
Ser							
Glu <sup>a</sup>	0.018	1.321	6.880	7.871	6.859	5.728	5.474
Pro	0.015	0.968	5.373	1.620	2.057	n.f.	n.f.
Gly	0.006	0.043	0.425	0.551	0.451	0.746	0.586
Ala	0.030	2.704	8.887	5.662	4.517	0.352	0.793
1/2-Cys	n.f. <sup>b</sup>	0.128	1.384	1.035	0.231	n.f.	n.f.
Val	0.007	0.493	4.422	3.766	n.f.	n.f.	n.f.
Met	n.f.	0.026	0.072	n.f.	n.f.	n.f.	n.f.
Ile	0.003	0.104	1.953	2.019	n.f.	n.f.	n.f.
Leu	0.003	0.146	1.396	1.207	2.527	4.978	3.314
Tyr	n.f.	0.039	0.268	0.287	0.394	0.622	0.771
Phe	n.f.	0.033	0.312	0.699	0.239	0.684	0.775

<sup>a</sup> Does not include any of those amino acids that may have been derived from asparagine or glutamine. <sup>b</sup> n.f. = not found. <sup>c</sup> The values are an average of two determinations.

Table III. Amino Acid Composition of Protein Hydrolysates at Various Maturity Stages (Micromoles per 10 Seeds)<sup>b</sup>

amino acid	days after opening of the flower						
	7	14	21	28	35	4	49
Lys	0.160	4.30	16.79	39.85	55.81	91.46	87.14
His	0.04	1.65	7.79	15.04	22.88	39.42	33.93
Arg	0.10	2.61	6.89	24.63	35.71	64.78	70.51
Asp	0.32	10.25	30.79	82.92	135.82	175.69	219.38
Thr	0.14	4.16	14.13	29.65	47.77	59.83	71.99
Ser	0.17	4.67	16.89	39.58	68.69	82.24	95.18
Glu	0.39	12.35	44.41	135.15	253.38	311.78	377.83
Pro	0.25	4.47	16.42	37.25	60.26	79.27	97.24
Gly	0.28	8.01	25.91	55.35	90.60	117.04	138.84
Ala	0.26	8.41	28.52	57.75	89.30	115.86	135.64
<sup>1</sup> / <sub>2</sub> -Cys <sup>a</sup>							
Val	0.22	6.39	23.57	25.15	76.36	96.53	110.74
Met	0.16	0.89	2.16	2.89	5.95	8.45	10.27
Ile	0.16	4.19	15.87	32.44	54.02	65.64	76.88
Leu	0.27	7.83	28.71	63.00	104.74	130.37	152.86
Tyr	0.08	2.31	8.53	6.34	28.50	34.38	40.87
Phe	0.11	3.25	12.21	37.27	71.04	89.15	112.60

<sup>a</sup> Values of <sup>1</sup>/<sub>2</sub>-Cys were not reported because of possible destruction due to acid hydrolysis. <sup>b</sup> The values are an average of two determinations.

Table II. The results showed marked changes in the free amino acid pool both quantitatively and qualitatively during various developmental stages. The concentration of most amino acids increased from 14 to 28 days followed by a rapid decline toward maturity. Leucine increased gradually up to 42 days followed by a slight decline at maturity. The maximum amount of storage protein accumulation in the seed occurred between 21 and 42 days, and thus was accompanied by quantitative changes in almost all the free amino acids in the pool. Most amino acids decreased in the free pool from 28 days to maturity. The changes in concentration of free amino acids observed may be due to their utilization in protein synthesis and also to differences in their metabolism and translocation. The amino acids which were not detected during the final stages of seed maturation, included valine, isoleucine, cysteine, proline, aspartic acid, serine, and threonine. Sulfur-containing amino acids, which are the most limiting in legumes, were present in very low concentration and were found only around 14–28 days of seed maturation. Since sulfur amino acids are the most limiting in legume proteins, it is likely that application of sulfur fertilizers might elevate their levels in the grains, thus increasing their availability for protein synthesis. Such a response to sulfur fertilization has earlier been reported for lupins by Blagrove et al. (1976) and Gillespie et al. (1978).

#### Amino Acid Composition of Protein Hydrolysates.

The amino acid composition of pigeon pea protein hydrolysates at various maturity stages is shown in Table III. Pigeon pea proteins were found to be rich in aspartic acid, glutamic acid, leucine, glycine, and alanine at all the stages of seed development. The present investigations also suggest that the pigeon pea proteins are extremely poor in the sulfur amino acids. These results indicate that the storage proteins of pigeon pea are similar to the seed proteins of yam bean and lime bean (Evans and Boulter, 1974) while slightly lower in lysine content as compared to chickpea storage proteins (Srivastava et al., 1981).

The failure to detect the sulfur-containing amino acids in the free pool, particularly during the final stages of seed maturation, might be one of the limiting factors for the

low sulfur content of pigeon pea proteins. It is thus important to characterize the biochemical impediments or inadequacies that restrict the synthesis of sulfur-containing amino acids during seed development in legumes.

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**Registry No.** Val, 72-18-4; Ile, 73-32-5; Cys, 52-90-4; Pro, 147-85-3; Asp, 56-84-8; Ser, 56-45-1; Thr, 72-19-5; Met, 63-68-3; Tyr, 60-18-4; His, 71-00-1.

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