Phytochemistry, Vol. 21, No. 2, pp. 297–299, 1982.
Printed in Great Britain.

0031-9422/82/020297-03$03.00/0

CHANGES IN STARCH, OLIGOSACCHARIDES AND SOLUBLE SUGARS IN DEVELOPING POD WALL AND SEED OF CHICKPEA*

U. SINGH and R. JAMBUNATHAN

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ICRISAT Patancheru P.O. 502 324, Andhra Pradesh, India

(*Received* 16 *June* 1981)

Key Word Index—*Cicer arietinum*; Leguminosae; chickpea; starch; oligosaccharides; sugars; pod wall; seed maturation.

Abstract—The accumulation of starch in the seed of chickpea accompanied by a decline in the pod wall during the early stages of development probably indicates that seed and pod wall did not compete with each other for starch accumulation. During the early stages of maturation, the reducing and non-reducing sugars showed a decline in seeds whereas non-reducing sugars decreased in the pod wall. The accumulation of the oligosaccharides raffinose, stachyose, and some other unidentified oligosaccharides was accompanied by a decline in the mono- and disaccharides in the developing seeds.

INTRODUCTION

The aim of this work was to measure the accumulation of sugars and starch in the pod wall and seed during development of the fruit of the nutritionally important legume, the chickpea (*Cicer arietinum*). Two cultivars, G-130 and L-550, were studied.

RESULTS AND DISCUSSION

Development of pod wall and seed

The changes in fr. and dry wt of the pod wall and seed for the two cultivars are shown in Figs. 1 and 2.

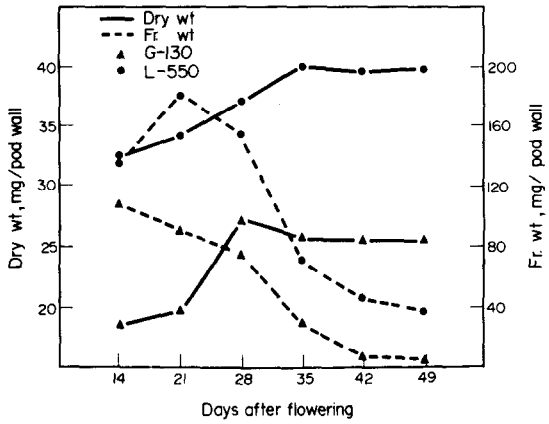

Fig. 1. Development of pod wall of chickpea cvs G-130 and L-550 at different stages of maturation.

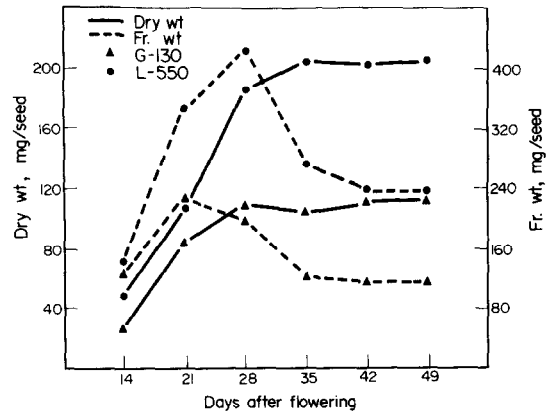

Fig. 2. Development of seed of chickpea cvs G-130 and L-550 at different stages of maturation.

Differences between the two cultivars are probably due to variation in the time taken for the seeds to mature. The observation that pod and seed matured at different rates agrees with data for french and soy beans [1].

Starch content of pod wall and seed

The starch content in the pod wall of both the cultivars declined between 14 and 21 days after flowering and then remained more or less constant up to maturity (Table 1). In case of the seed, starch content increased up to 28 days after flowering and then showed a slight decrease during the later stages of maturation in G-130, and in L-550 did not change until maturity. Although an increase in the accumulation of starch of seed was accompanied by a

*Submitted as J. A. No. 172 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Table 1. Amounts of starch, reducing and nonreducing sugars in pod wall and seed of chickpea at different stages of maturation*

Days after flowering	Pod wall			Seed		
	Starch	Reducing sugars	Non-reducing sugars	Starch	Reducing sugars	Non-reducing sugars
14 a	16.6	1.6	10.0	46.1	4.4	9.9
b	26.1	1.6	4.7	48.0	7.4	11.1
21 a	12.1	1.3	2.7	49.2	0.2	4.8
b	11.8	2.0	1.0	58.7	1.0	6.6
28 a	12.0	1.1	2.6	51.2	0.2	4.8
b	10.8	1.5	1.0	59.8	0.4	6.2
35 a	12.6	1.1	2.5	49.8	0.1	4.7
b	11.2	1.3	1.0	59.3	0.2	6.1
42 a	12.8	1.1	2.3	49.5	0.1	4.5
b	12.2	1.0	0.9	57.4	0.1	6.0
49 a	12.3	1.0	2.3	48.5	0.2	4.6
b	12.0	0.8	0.9	58.7	0.1	6.1

*Results are expressed as per cent dry wt and are averages of two independent determinations.

a: cv. G-130; b: cv. L-550.

decline in pod wall, it is not possible to say whether the starch content of the pod wall was mobilized and translocated to the seed. However, earlier studies with bean cultivars, using labelled compounds have shown that the seed did not depend on the pod for photosynthetic product [2]. It appears from the present study that pod wall and seed did not compete with each other as far as the accumulation of starch is concerned.

Reducing and non-reducing sugars

The difference between the total soluble sugars and reducing sugars was assumed to comprise non-reducing sugars and these are reported in Table 1. Percentages of reducing and non-reducing sugars of the seed decreased in both cultivars during early stages of development but the decline was greater in the case of the reducing sugars than that of the non-reducing sugars. The decline of reducing sugars in the seed might suggest that these sugars are utilized for the accumulation of non-reducing sugar and starch during development. The reducing and non-reducing sugars of the pod wall changed in a different way from that of the seed during the course of development. During the early stages of maturation, non-reducing sugars decreased remarkably in the pod wall. The reducing sugars of the pod wall did not change much in the early stages, but a slight decrease was observed in the later stages of maturity (Table 1).

In the case of the seed, the percentage of total soluble sugars declined during the early stages of maturation indicating that these sugars were utilized for the accumulation of starch which increased during the same period. However, the decline in the total soluble sugars of the pod wall could have occurred for two possible reasons: (1) mobilization and translocation of these sugars to the seed, and (2) utilization of these sugars for the synthesis of carbohydrates other than starch.

The sugars, fructose, glucose, sucrose, raffinose, stachyose and at least two other unidentified oligosaccharides, account for *ca* 95% of the total soluble sugars of chickpea flour [3]. Using the TLC it was possible to demonstrate the relative changes in the concentrations of these sugars. Stachyose and another unidentified oligosaccharide were estimated together as they could not be resolved completely. Besides these, one more unidentified oligosaccharide was present in chickpea but was not measured because of unavailability of a proper standard. The sugars of the pod wall at different stages could not be measured quantitatively because these sugars failed to resolve satisfactorily by both the TLC and PC. As shown in Table 2, fructose, glucose and sucrose, were the predominant sugars of the seeds in the early stages of maturity. The concentration of these sugars slowly declined during the later stages of development in both cultivars. Raffinose and stachyose, with the unidentified oligosaccharides, were absent in the samples obtained at 14 days after flowering in the case of cv L-550, and in samples of 14 and 21 days after flowering in the case of cv G-130. These oligosaccharides appeared in samples 21 days after flowering and their concentration increased as the seed matured. These results are in agreement with the findings of earlier workers [4, 5] who have reported that raffinose accumulated only during the later period of seed development. A rapid increase in the concentration of stachyose and raffinose was observed between 21 and 35 days after flowering. The increase in the concentration of the oligosaccharides was accompanied by a decrease in the concentration of glucose, fructose, and sucrose, during the early stages of maturation. It is possible that these mono- and disaccharides are utilized for

Table 2. Amounts of mono-, di-, and oligo-saccharides of chickpea seed at different stages of maturation*

Days after flowering	Cv. G-130				Cv. L-550			
	Glucose + fructose	Sucrose	Raffinose	Stachyose + unidentified oligosaccharide	Glucose + fructose	Sucrose	Raffinose	Stachyose + unidentified oligosaccharide
14	29.7	43.5	nd	nd	35.4	49.7	nd	nd
21	23.3	43.0	nd	nd	13.7	32.2	2.8	10.4
28	8.9	31.9	9.3	14.1	9.3	25.7	4.7	18.3
35	4.4	24.4	11.8	29.5	4.5	21.6	9.3	30.5
42	nd	20.9	13.7	40.8	nd	19.2	11.5	29.7
49	nd	20.4	13.5	39.9	nd	19.6	12.7	31.5

*Results are expressed as per cent of total soluble sugars and are averages of two independent determinations.
nd—not determined.

the synthesis of oligosaccharides during the course of development.

EXPERIMENTAL

The chickpea cvs G-130 and L-550 used in the present study were grown during 1978–79 under normal cultural practices in the experimental plots of the Pulse Physiology Program at ICRISAT Center, near Hyderabad, India. Plants that flowered on the same day were chosen at random and the flowers were tagged. Samples 14, 21, 28, 35, 42 and 49 days after flowering were collected and chilled in ice. Sampling was done during the period between 16 Jan. and 20 Feb. 1979, for cv. L-550, and between 30 Jan. and 6 Mar. 1979, for cv. G-130 because these cultivars differed in their maturation period. Seeds were removed from the pods, and seed and pod wall samples were freeze-dried. The freeze-dried samples were ground to pass through a 100-mesh sieve. The ground samples were defatted in a Soxhlet apparatus using hexane.

Total soluble sugars and starch content. Soluble sugars were extracted with 80% EtOH in a Soxhlet apparatus and determined by the PhOH–H_2SO_4 method [6]. Starch was determined by enzymatic hydrolysis as described earlier [7]. The procedures followed produced results with s.e.s ± 0.14 and ± 1.63 for per cent soluble sugars and per cent starch, respectively.

Determination of reducing sugars. Extracts of soluble sugars were analysed for reducing sugars according to ref. [8] with a slight modification. To 1 ml of sugar soln an equal vol. of Cu reagent was added and the samples heated for 15 min at 100° and then cooled. Arsenomolybdate reagent (1 ml) was added and the soln diluted to 10 ml. After standing for 15 min, the *A* was read at 500 nm. Sample blanks were run in a similar way. Solns of glucose (0.1–0.6 mg) were used for prepn of the standard curve. The s.e. of the procedure was ± 0.02 for per cent reducing sugars. The difference between the total soluble sugars and reducing sugars was assumed to comprise non-reducing sugars.

Separation of sugars by TLC. TLC was carried out on 500 μm-thick Si gel G plates. The solvent was $CHCl_3$–HOAc–H_2O (6:7:1). The separated sugars were detected by spraying with an aniline–diphenylamine soln which was prepared by mixing 5 vol. of 1% aniline and 5 vol. of 1% diphenylamine in Me_2CO with 1 vol. of 85% H_3PO_4. Equal concns of sugars were applied for each stage of maturity. The sugars in the extracts were determined according to the procedure described earlier. The sugars sepd by TLC were scanned in a densitometer and the area of the peaks and their concns were estimated by comparison with data for the respective standard sugars obtained under similar conditions. Glucose and fructose did not separate well and therefore were analysed together. The mean coefficient of variability for the different sugars ranged between 5.6 and 12.4%.

Acknowledgements—The authors are grateful to Dr. N. P. Saxena, Physiologist, for his help during the collection of samples from his experimental field. The technical assistance of Mr. N. Subrahmanyam is also gratefully acknowledged.

REFERENCES

1. Onebedeanx, B. and Chollet, R. (1975) *Plant Physiol.* **55**, 745.
2. Oliker, M., Mayber, A. P. and Mayer, A. M. (1978) *Am. J. Botany* **65**, 366.
3. Lineback, D. R. and Ke, C. H. (1975) *Cereal Chem.* **52**, 334.
4. Rockland, L. B. (1961) in *Proc. Fifth Dry Bean Research Committee Conference*, Denver, CO, December 1951.
5. Korytnk, W. and Metzler, E. A. (1962) *Nature (Lond.)* **195**, 616.
6. Debois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Analyt. Chem.* **28**, 350.
7. Singh, U., Jambunathan, R. and Narayanan, A. (1980) *Phytochemistry* **19**, 1291.
8. Nelson, N. (1944) *J. Biol. Chem.* **153**, 375.