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Changes in Some Enzymes of Carbohydrate Metabolism in Developing Pod and Seed of Chickpea (*Cicer arietinum*)

By

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With 3 Figures

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Summary

SETIA N. & MALIK Ch. P. 1985. Changes in some enzymes of carbohydrate metabolism in developing pod and seed of chickpea (*Cicer arietinum*). — Phyton (Austria) 25 (1): 93—99, 3 figures. — English with German summary.

Changes in carbohydrates (total soluble sugars, starch) and activities of some enzymes of carbohydrate metabolism were recorded in developing pod and seed of chickpea (*Cicer arietinum*) over the period of 7—42 days after anthesis (DAA). Initially carbohydrates accumulated rapidly in pod towards the maximum at 28 DAA. Changes in activity profile of amylases, invertase and sucrose synthetase (both synthesis and cleavage enzymes) closely matched with the changes in reserve substances both in developing pod and seed. Activities of glucose-6-phosphate dehydrogenase and pyruvic kinase also exhibited some correlation with the developing organs.

Zusammenfassung

SETIA N. & MALIK Ch. P. 1985. Veränderung einiger Enzyme des Kohlenhydratstoffwechsels in Hülsen und Samen der Kichererbse (*Cicer arietinum*) während der Entwicklung. — Phyton (Austria) 25 (1): 93—99, 3 Figuren. — Englisch mit deutscher Zusammenfassung.

Die Veränderungen im Kohlenhydratgehalt (gesamter löslicher Zucker, Stärke) und der Aktivitäten einiger Enzyme des Kohlenhydratstoffwechsels in Hülsen und Samen der Kichererbse (*Cicer arietinum*) wurde über einen Zeitraum von 7—41 Tagen nach der Anthese verfolgt. Zunächst reichern

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sich in der Hülse Kohlenhydrate mit großer Geschwindigkeit zu einem Maximum am 28. Tag nach der Anthese an. Während der Entwicklung von Hülse und Samen stehen die Änderungen der Amylase-, Invertase- und Rohrzuckersynthetase-Aktivitäten mit denen der Reservestoffe in engem Zusammenhang. Auch die Aktivitäten der Glucose-6-phosphatdehydrogenase und der Pyruvatkinase stehen mit dem Entwicklungszustand der Organe in einer gewissen Beziehung.

(Editor transl.)

1. Introduction

The legume fruit is characterized by precocious growth of pod (Carr & Skene 1961). However, considerable metabolic changes continue to take place even after pod attains full size. These metabolic changes coincide with the rapid growth of seeds. The development of legume seed has been studied from various angles (Bisson & Jones 1932, McKee *et al.* 1955, Bain & Mercer 1966, Raacke 1957, Flinn & Pate 1968, Rauf 1978). However, detailed biochemical studies on the role of pod in seed development in chickpea (*Cicer arietinum*) are not available. The present communication describes changes in carbohydrates (sugars, starch) and some enzymes of carbohydrate metabolism during its pod and seed development. Biochemical changes in developing pod and seed are correlated.

2. Material and Methods

Developing fruits at 7, 14, 21, 28, 35 and 42 days after anthesis (= DAA) were collected from chickpea plants (*Cicer arietinum* L. cv. C-214), grown in the experimental area of Department of Botany. Fruits split into pods and seeds were used for biochemical analysis separately. Soluble sugars and starch were determined following the method of Minamikawa (1979).

For assaying activities of various enzymes, samples were hand homogenized at 0—4° C using phosphate buffer pH 7.4 for amylases and Tris-HCl buffer pH 7.4 containing 0.5 M mercapto-ethanol for other enzymes. Following enzymes were analyzed according to the method of authors given in paranthesis: α-amylase (Murata *et al.* 1968), β-amylase (Duffus & Rosie 1973), invertase (Sumner 1935), sucrose synthetase (Hawker 1971), starch phosphorylase (Turner & Turner 1957), glucose-6-phosphate dehydrogenase (Komamine & Shimizu 1957) and pyruvic kinase (Duggleby & Dennis 1973).

3. Results

3.1. Changes in content of sugars and starch

The content of total soluble sugars and starch of a chickpea pod first reached maximum level at 28 DAA (Table 1). In seed these sub-

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Table 1

Changes in the endogenous level of total soluble sugars and starch (µg/organ) in developing fruit of chickpea

Days after anthesis (DAA)	Pod		Seed	
	Soluble sugars	Starch	Soluble sugars	Starch
7	137	30	21	70
14	96	180	33	180
21	308	800	154	240
28	315	1880	178	800
35	196	1510	215	1830
42	42	440	539	2660

stances increased continuously with the advancement of development. A rapid accumulation of starch occurred in seed during 21 to 42 days stages. The decline in the level of these substances in pod coincided with the active period of accumulation of reserves in the seeds.

3.2. Changes in enzyme activity

The activity of α- and β-amylases was very low initially (7 DAA) in both pod and seed (Fig. 1). In pod the activity of these enzymes increased until 28 days stage and remained sufficiently high during later stages. In seed the activity of these enzymes remained low during

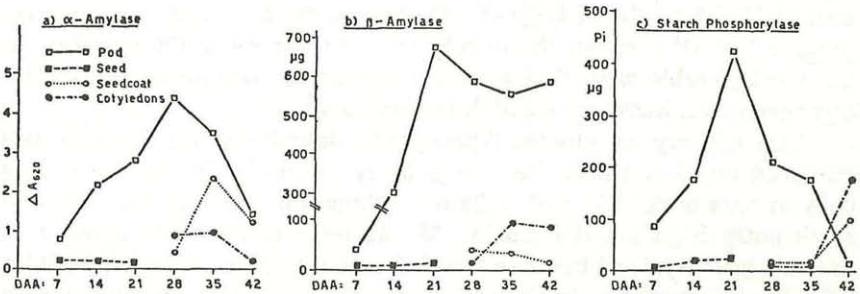

Fig. 1. Changes in activities of α-amylase (a), β-amylase (b) and starch phosphorylase (c) in pod (empty squares, straight lines), seed (black squares, broken lines), seedcoat (empty circles, dotted lines) and cotyledons (black circles, dash-dotted lines) of developing fruit of chickpea. Abscissa: DAA = days after anthesis. Ordinates: a) difference of absorptivity (λ = 620 nm) per hour and organ; b) µg maltose formed per hour and organ; c) µg inorganic phosphorous released within 1 hour per organ.

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7-21 DAA followed by a rise at 35 DAA. Starch phosphorylase in the pod attained a peak activity at 21 DAA and then declined. Seeds exhibited very low activity of this enzyme till 35 DAA followed by a slight increase thereafter (Fig. 1).

Activity profiles of invertase and sucrose synthetase (both synthesis and cleavage enzymes) in developing pod and seed are shown in Fig. 2. In pod the activity of invertase was high during 7—14 DAA. Another peak of enzyme activity was noticed at 28 DAA. Unlike pod, invertase activity was extremely low in seeds.

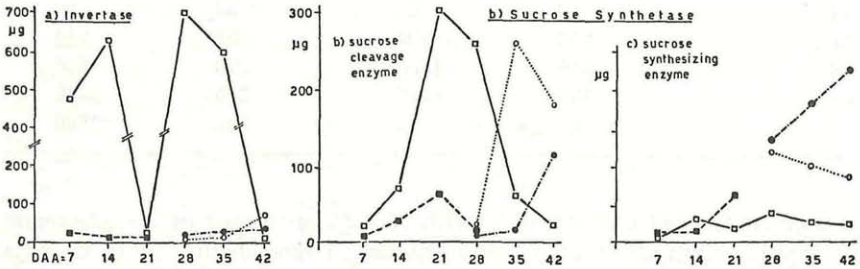

Fig. 2. Activities of invertase and sucrose synthetase in pod, seed, seedcoat and cotylendons of developing fruit of chickpea. Symbols and signatures see Fig. 1. Abscissa: DAA = days after anthesis. Ordinates: a) and b) = µg glucose formed per hour and organ; c) = µg sucrose formed per hour and organ

The activity of sucrose synthetase (sucrose cleavaging enzyme) in pod was low initially (7 DAA) and then reached maximum level at 21 DAA and remained sufficiently high at 28 DAA. Compared with the cotyledons the activity of this enzyme was sufficiently high in seed coat at 35 DAA. The activity of sucrose synthesizing enzyme in pod remained low all through the development but in seeds its activity was quite comparable with that of sucrose cleavage enzyme up to 21 DAA and thereafter, increase was high in cotyledons.

The activity of glucose-6-phosphate dehydrogenase in both pod and seed was low till 28 DAA (Fig. 3). A sharp rise in activity of this enzyme was noticed in pod at 35 days stage. Cotyledons also exhibited sufficiently high activity during 35—42 days stages. The activity of pyruvic kinase in pod increased sufficiently attaining a peak at 21 DAA (Fig. 3). However, seeds showed low activity of this enzyme during this period followed by a rise thereafter.

4. Discussion

The data on accumulation of starch and sugar in pod and changes in enzyme activity of invertase and sucrose cleavage enzyme indicate that synthesis and deposition of reserves is accompanied by meta-

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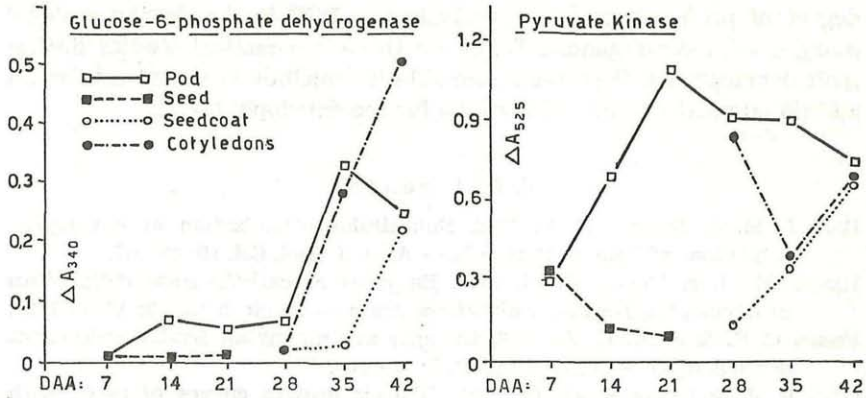

Fig. 3. Activities of glucose-6-phosphate dehydrogenase and pyruvic kinase in pod, seed, seed coat and cotyledons of developing fruit of chickpea. Symbols and signatures see Fig. 1. Abscissa: DAA = days after anthesis; Ordinates: a) = differences of absorptivity (λ = 340 nm) per minute and organ; b) = difference of absorptivity (λ = 525 nm) per hour and organ.

bolic processes probably to meet the energy requirements for the various anabolic processes.

The activity of invertase and sucrose cleavage enzyme is quite high in pod during 7—28 days stages. These enzymes are known to be involved in breakdown of sucrose which is a predominant form of carbon translocated from vegetative parts to the developing reproductive structures. During development, the translocated sucrose is converted into glucose, fructose and sugar nucleotides (Baxter & Duffus 1973, Hawker 1971). On the other hand, low activity of sucrose synthesizing enzyme is observed in pod throughout its development and in seed during 7—21 DAA. In the cotyledons, high activity of this enzyme during later stages of development coupled with low activity of invertase and sucrose cleavage enzyme suggested the role of this enzyme in resynthesis of sucrose from monosaccharides. The pattern of sucrose breakdown and synthesis is comparable to that reported for *Hordeum* (Duffus & Rosie 1975) and pea (Turner & Turner 1957).

Based on the activity profile of pyruvic kinase during early stages of pod and later stages of seed development high glycolytic activity is apparent. It is well known that glycolysis in conjunction with TCA-cycle supplies ATP and precursors for various biosynthetic processes. High activity of glucose-6-P-dehydrogenase during later stages of both pod and seed development points towards an active operation of pentose phosphate pathway (PPP). This pathway is generally associated with need for high reducing power for high energy requiring organogenetic processes (Dhindsa *et al. 1979*). According to Ting (1981) the

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degree of predominance of glycolysis and PPP in developing systems changes with development. Based on these biochemical studies during fruit development, it seems reasonable to conclude that pod acts as an additional nutrient-supplying organ for the developing seed.

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