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Photosynthetic Contribution of Podwall in Seed Development of Chickpea (*Cicer arietinum* L.)

I S SHEORAN and RANDHIR SINGH
Department of Chemistry and Biochemistry, Haryana Agricultural University, Hisar 125 004

Photosynthetic contribution of developing chickpea pod wall was studied during seed development in twelve genotypes. Carbon dioxide exchange studies carried out with IRGA showed that podwall fixed net CO_2 in light during the early phase of seed development. This contributed about 20% to seed dry matter. However, genotypic differences were observed. Most of the genotypes contributed between 14 to 24% to seed dry weight. Presence of stomata on the outer surface, fixation of labelled carbon dioxide from atmosphere and presence of enzymes of CO_2 fixation and metabolism further confirmed that podwall fixed CO_2 photosynthetically. Puncturing of pod up to day 14 after anthesis did not allow seed to develop. However, if puncturing was done after this stage, there was a net loss of about 29% in seed dry matter.

Key Words: Chickpea, Pods, Stomata, CO_2 fixation, Dry matter

Introduction

Studies carried out in our laboratory have shown that reproductive parts (pods) of pigeonpea and chickpea have higher activities of PEP-carboxylase and related enzymes of C_4-metabolism compared to the enzymes of the PCR cycle (Luthra et al. 1983, Singal et al. 1986). A general role ascribed to legume pods is to refix the CO_2 released during respiration or photorespiration, as little net photosynthesis has been shown to occur in pods (Croockston et al. 1974, Quebedeaux & Chollet 1975). On the contrary, pod photosynthesis can improve the economy of carbon usage in a fruit by 16 to 20% (Flinn et al. 1977). Hence, a search for more desirable structural and biochemical traits in legume pods might lead to further improvements in fruit economy and ultimately in seed yield. In view of above, we have studied the photosynthetic contribution of chickpea podwall in seed development.

Materials and Methods

Plant Material: Twelve chickpea genotypes were raised in the fields of the Pulse Section of Haryana Agricultural University, Hisar, by following the recommended agronomic practices. Fully opened flowers were tagged on the day of anthesis.

Photosynthetic Contribution

On 3rd and 18th days after anthesis, pods of 12 genotypes were covered with aluminium foil in such a way that no light could reach the pods. In all, five replicates of 10 pods each were covered in each treatment. At the time of harvest, control as well as covered pods were removed and their dry weights recorded.

Gas Exchange Studies

These were conducted only in one genotype (H-75–35). At 3 days interval, 15 pods were sampled (three replicates of five pods each) and their CO_2 exchange was monitored both in dark and light with the help of IRGA (Model 225/MK 3).

$^{14}CO_2$ Feeding

During the period of rapid seed growth, $^{14}CO_2$ feeding was done both from outside and inside of the podwall. In the first case, the intact pods were enclosed in a transparent polyethylene bag and 1ml of labelled CO_2 was injected. Incubation was done for 20 sec and pods were sampled at 0, 60, 120, 300 sec and 24 and 48 hr after feeding. Immediately, the pods were separated

into pod-walls and seeds and killed in boiling 80% ethanol. Ethanol soluble compounds were extracted and estimated by Liquid Scintillation Counter as described by Singhal et al. (1986).

In another set, 0.1ml of labelled CO_2 was injected inside the pod cavity with the help of a syringe and the hole was immediately sealed with vaseline. Pods were then sampled at 30, 60, 300 sec, 1 hr and 48 hr after feeding. Pods were separated and processed as described above.

Pod Puncturing

Tagged pods were punctured at 7, 14, 21 and 28 days after anthesis with the help of a 2mm needle in such a way that no damage occurred to the translocatory system. Ten such pods were harvested after 7 days till maturity and their fresh and dry weights recorded.

Microscopic Observation

Both outer and inner surfaces of pod-wall were examined under a light microscope for stomata.

Results

Carbon dioxide studies showed that pod fixed net CO_2 in light up to day 21 after anthesis. The maximum fixation was observed on day 18 after anthesis (100μg CO_2 $pod^{-1}hr^{-1}$). However, in dark, there was a net loss of CO_2 (200μg CO_2 $pod^{-1}hr^{-1}$). A continuous increase in dark loss was observed up to day 21 after anthesis. At maturity, CO_2 loss during light (210μg CO_2 $pod^{-1}hr^{-1}$) was even more than the loss in dark (115 μg CO_2 $pod^{-1}hr^{-1}$).

Microscopic examination of the pod-wall revealed stomata on the outer surface which were similar to leaf stomata in size and shape. However, inner surface of the pod-wall did not have any stomata.

Seed yield was reduced to different extents in different genotypes, when the pod was covered with aluminium foil (table 1). Variety H-86–108 hardly showed any reduction. Maximum reduction was observed in H-86–60 (20.8%). In nine varieties, the reduction was between 14 and 21%. When pods were covered on 18 days after anthesis, only two varieties namely H-208 and H-86–78 showed 17 and 24% reduction, respectively. Other varieties showed little reduction in seed dry weight.

When $^{14}CO_2$ was fed from outside, label was observed in the seeds only after 5 min of feeding (table 2). At 24 hr about 40% of the label moved to the seeds which increased to about 50% at 48 hr. However, maximum (up to 90%) fixation occurred in podwall when the pods were fed through internal cavity. Seeds fixed only a small amount of CO_2 (10%). Even after 48 hr of feeding, seeds showed about 33% labelling (table 2).

Table 1 *Effect of pod cover on seed yield in chickpea genotypes*

Genotypes	Control	Seed dry weight (mg/pod) (after anthesis)	
		3 days	18 days
H-75-35	286	229 (19.9)*	281 (1.7)
H-86-45	160	138 (13.7)	154 (3.7)
H-86-59	180	157 (12.7)	170 (5.5)
H-86-60	221	175 (20.8)	212 (4.0)
H-86-61	236	194 (17.8)	206 (12.7)
H-86-69	152	126 (17.1)	137 (9.8)
H-86-78	223	197 (11.6)	168 (24.6)
H-86-89	190	163 (14.2)	167 (12.1)
H-86-91	207	174 (15.9)	194 (6.2)
H-86-108	231	229 (0.8)	227 (1.7)
H-86-131	231	199 (13.8)	204 (11.6)
H-208	189	162 (14.2)	156 (17.4)

*Values in parenthesis is per cent change over control

Table 2 *Photosynthetic $^{14}CO_2$ fixation by 20 days old chickpea pods*

Time after start of feeding (sec)	Total ^{14}C-recovered in ethanol soluble fraction (cpm)	
	In Podwall	In Seeds
External Feeding		
20*	3865 (98%)	81 (2%)
60	4019 (98%)	90 (2%)
120	4692 (98%)	120 (2%)
300	4766 (92%)	416 (8%)
24 hr	1780 (59%)	1220 (41%)
48 hr	1210 (49%)	1260 (51%)
Internal feeding		
30	2960 (90%)	329 (10%)
60	8826 (94%)	650 (6%)
300	12727 (91%)	1250 (9%)
1 hr	15646 (91%)	1505 (9%)
48 hr	2701 (67%)	1331 (33%)

*$^{14}CO_2$ was supplied externally for 20 sec and then the pods were exposed to natural conditions

In pods punctured on 7th day, there was no further pod development. Pods were completely dried out within a week after puncturing. Similarly, in pods punctured on the 14th day, there was no further development (table 3). But with subsequent puncturing, pod development occurred at a faster rate and seed matured at least a week before compared to the unpunctured pods. This resulted in a final reduction of 29% in pod dry weight. At this stage, the pod had the capacity to close the puncture to some extent by repair mechanism. Similar was the case when the pods were punctured at 28 days after anthesis. However, there was no effect when puncturing was done on 35th day.

Discussion

A number of workers have reported that legume pods fix very little net CO_2 in light (Croockston et al. 1974, Quebedeaux & Chollet 1975), though they have chlorophyll. Chickpea pods are also having chlorophyll and stomata on the outer surface (data not given). Interestingly, in the present case, in the presence of light, there was net CO_2 fixation which increased up to day 18 after anthesis. This clearly showed that podwall of chickpea fixed atmospheric CO_2 photosynthetically during the early phase of pod development. This was further confirmed by labelling studies. $^{14}CO_2$ was fixed by the podwall when fed externally (table 2). Similar fixation has been observed earlier in field pea (Flinn & Page 1970) and chickpea (Singh & Pandey 1980). Fixed CO_2 by the pod wall was transported to seeds with a lag of about 5 min (table 2). In chickpea pods, about half of the fixed carbon by pods was translocated to the seeds on 9th day after anthesis but very little translocation was observed during later stages of growth (Singh & Pandey 1980). However, Khanna-Chopra and Sinha (1976) observed that podwall translocated more photosynthates than leaves during later stages of pod development in pea.

Presence of photosynthetic enzymes i.e. RuBP carboxylase and other enzymes of PCR cycle (Singal et al. 1986) in podwall further stregthens our view that net CO_2 fixation occurs in pods. The presence of these enzymes have also been shown in other legumes.

Podwall in the present case contributed about 20% of photosynthates towards their seed dry weight (table 1). This contribution varied depending upon the genotype (14 to 24%). This is contrary to the results reported by Singh and Pandey (1980). In pea, pod photosynthesis could improve the economy of carbon usage by about 16 to 20% (Flinn et al. 1977).

Inner layers of pea pods have been shown to be the most active site for refixation of respired CO_2 (Flinn et al. 1977). Chickpea podwall also fixed $^{14}CO_2$ when fed through internal cavity. Podwall was far more efficient (90%) in CO_2 fixation than the seeds (10%).

Podwall of chickpea seems to act as an impermeable barrier to internal CO_2 thereby accumulating high concentration of CO_2 (2.6%) in the pod cavity, which was maximum during rapid phase of seed growth. Interestingly, seeds did not develop when the podwall was made permeable by puncturing and CO_2 was allowed to escape, particulary during the early phase of pod development. The complete failure of pod development when punctured during earlier phase, seems to be due to the loss in turgor in addition to CO_2 loss. It appears that internal CO_2 refixation by pods accounts for about 29% towards seed dry weight. This has an agricultural importance, as this crop is mainly

Table 3 *Effect of pod puncture on pod weight (mg/pod) in chickpea*

Time of pod puncture (Days after anthesis)	Days after puncture					
	7		14		21	
	C	P	C	P	C	P
7	75 ± 6.4	2 ± 0.01	135 ± 11.6	—	215 ± 18.6	—
14	135 ± 11.6	38 ± 4.3	215 ± 18.6	28 ± 3.5	302 ± 30.6	32 ± 2.8
21	215 ± 18.6	197 ± 13.3	302 ± 30.6	274 ± 22.8	399 ± 28.7	280 ± 30.1
28	302 ± 30.6	275 ± 21.7	399 ± 28.7	276 ± 17.3	—	—
35	399 ± 28.7	409 ± 31.4	—	—	—	—

C, Control *P*, Punctured

damaged by pod borer (*Heliothis)* which drills a hole in the podwall. Therefore, appearance of pod borer relatively earlier during the pod development period might be more damaging to grain yield than their late appearance.

In conclusion, podwall of chickpea plays an important role in the seed development. However, genotypic differences occur in this respect.

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