

Relationship of developing pods with photosynthetic characteristics of leaves in chick pea

ANIL GROVER and S K SINHA*

Plant Physiology and Biochemistry Laboratory, Water Technology Centre, Indian Agricultural Research Institute, New Delhi 110 012, India

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Abstract. Net photosynthesis rate, ribulose-1,5-bisphosphate carboxylase activity, ribulose-1,5-bisphosphate carboxylase protein and total chlorophyll content in leaves which subtend the pods vis-a-vis the pattern of pod, podwall and seed growth were analyzed in chick pea. Three sets of plants namely control, deflowered and depodded were maintained. Accompanying the initiation of pod development in control plants, there was a decline in these photosynthetic characteristics in leaves of control as well as deflowered plants. The senescence rate was higher in control plants compared to plants from which flowers or pods had been removed. The early pod growth which was mainly constituted by podwall growth, was accompanied by a higher decline in leaf net photosynthesis rate. Ribulose-1,5-bisphosphate carboxylase activity and ribulose-1,5-bisphosphate carboxylase protein decreased predominantly at the later stage of pod growth, which was mainly constituted by higher seed growth. Loss of chlorophyll was also higher at later stages of pod growth. It is suggested that both nutrient remobilization and hormonal action are probably involved during monocarpic senescence in chick pea.

Keywords. Chick pea; net photosynthesis rate; pod growth; RuBP carboxylase; senescence; subtending leaves.

1. Introduction

The onset of flowering in monocarpic plants initiates leaf senescence (Lindoo and Nooden 1976). It has been argued that the remobilization of nutrients from the leaves to the developing grains is the most plausible explanation for monocarpy (Malik and Berrie 1975; Sinclair and deWit 1976). In particular, nitrogen remobilization in crops having high nitrogen: carbon ratio in grains has been proposed to be the crucial event, leading to leaf senescence (Sinha 1977; Thomas and Stoddart 1980). However, it has also been suggested that the developing pods induce senescence of the subtending leaf by some hormonal signal, which may or may not have any relationship with the nutrient remobilization (Nooden *et al* 1978). We have attempted to characterise the senescence-associated changes in several photosynthetic components such as net photosynthesis rate, ribulose-1,5-bisphosphate carboxylase activity, ribulose-1,5-bisphosphate carboxylase protein and total chlorophyll content in subtending leaves and the parallel changes in dry matter accumulation characteristics in podwall and seeds in chick pea. Flower and pod removal treatments were practiced in order to examine the behaviour of leaf metabolic parameters in the absence of developing pods. The comparative data obtained in the present study on flowering—onwards loss of net photosynthesis rate and ribulose-1,5-bisphosphate carboxylase enzyme and accumu-

* To whom all correspondence should be addressed.

lation of dry matter in pods indicate that leaf senescence in chick pea probably involves both nutrient remobilization and hormonal interactions.

2. Materials and methods

Leaves from the top 8 nodes of the main axis of field grown chick pea (*Cicer arietinum* L. var. JG-62; Bengal gram) were analysed throughout the present investigation (figure 1). Seeds were inoculated with proper Rhizobia culture prior to sowing. Flowering plants were tagged and in subsequent course 3 sets were maintained. In the 1st set of plants called as deflowered (Df) plants, flowers were removed at regular intervals from the specified nodes and thus pod formation was completely prevented. Pods were removed from the top 8 nodes after 10 days of flowering from the plants of 2nd set, referred to as depodded (Dp) plants. Pod formation was left undisturbed in control (C) plants. Sampling was done at flowering, young pod (10 days after flowering) and mature pod (20 days after flowering) stages.

Pods harvested from these nodes from the control plants were randomized. Dry matter accumulation in podwall and seeds was analyzed separately after drying the pods at 80°C for 48 hr. Rate of net photosynthesis was determined using intact plants by feeding $^{14}\text{CO}_2$ as described earlier (Grover *et al* 1985). The system consisted of an air tight, 4 mm thick, transparent, plexiglass chamber ($36 \times 18 \times 12 \text{ cm}^3$), connected with a battery operated air circulating pump at a speed of 1450 revolutions per min. Shoots to be fed were placed in the chamber and $^{14}\text{CO}_2$ generated by adding a few drops of 1 M HCl to $3.77 \times 10^2 \text{ KB NaH}^{14}\text{CO}_3$ ($204 \times 10^4 \text{ KBq m mol}^{-1}$) was circulated into it for 2 min. Fixed radioactivity was extracted in 80, 50 and 30% ethanol and finally in water. All extracts were combined and an aliquot was counted in a Scintillation spectrometer. Scintillation mixture contained 4 g 2,5-diphenyloxazole (PPO) and 100 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in 1 litre toluene.

Crude enzyme extract for assaying ribulose-1,5-bisphosphate (RuBP) carboxylase activity was prepared by the method of Marco *et al* (1979) in 0.1 M Tris-HCl buffer, pH 8.3, containing 20 mM MgCl_2 and 1 mM reduced dithiothreitol (DTT). Partial purification and activation of the enzyme was done by passing the supernatant obtained after centrifugation through a sephadex G-25 column (15 cm length \times 1.7 cm inner diameter) previously equilibrated with activation buffer (extraction buffer containing 10 mM NaHCO_3). Activity was assayed following Bjorkman (1968) with the only modification that reduced glutathione was replaced by reduced cysteine in the reaction mixture. The composition of the reaction mixture was as follows (in μmol): NaHCO_3 2.5; Cysteine 1.25; EDTA 0.1; MgCl_2 2.5 and Tris-HCl 10.0 (pH 8.3). Reaction was carried out at 25°C for 1 min using 0.2 ml of reaction mixture, 0.05 ml of enzyme extract, 0.1 ml of RuBP ($1.5 \mu\text{mol ml}^{-1}$) and 0.1 ml of $\text{NaH}^{14}\text{CO}_3$ solution ($1.88 \times 10^2 \text{ KBq ml}^{-1}$), ($104 \times 10^4 \text{ KBq mol}^{-1}$) and fixed ^{14}C activity was determined.

Soluble proteins, extracted in chilled 0.05 M Tris-HCl buffer (pH 7.5) containing 5 mM reduced cysteine and 5 mM EDTA, in 1:3 w/v ratio, were subjected to discontinuous polyacrylamide gel electrophoresis following Davis (1964). After polymerizing the gels, an aliquot containing 200 μg of the protein sample, determined by the method of Lowry *et al* (1951), was layered on the top of each gel. Electrophoresis was carried out in cold by applying 3 milli ampere current per tube. Staining was done for 30 min with 1% amido black solution, made in 7% acetic acid. After destaining the

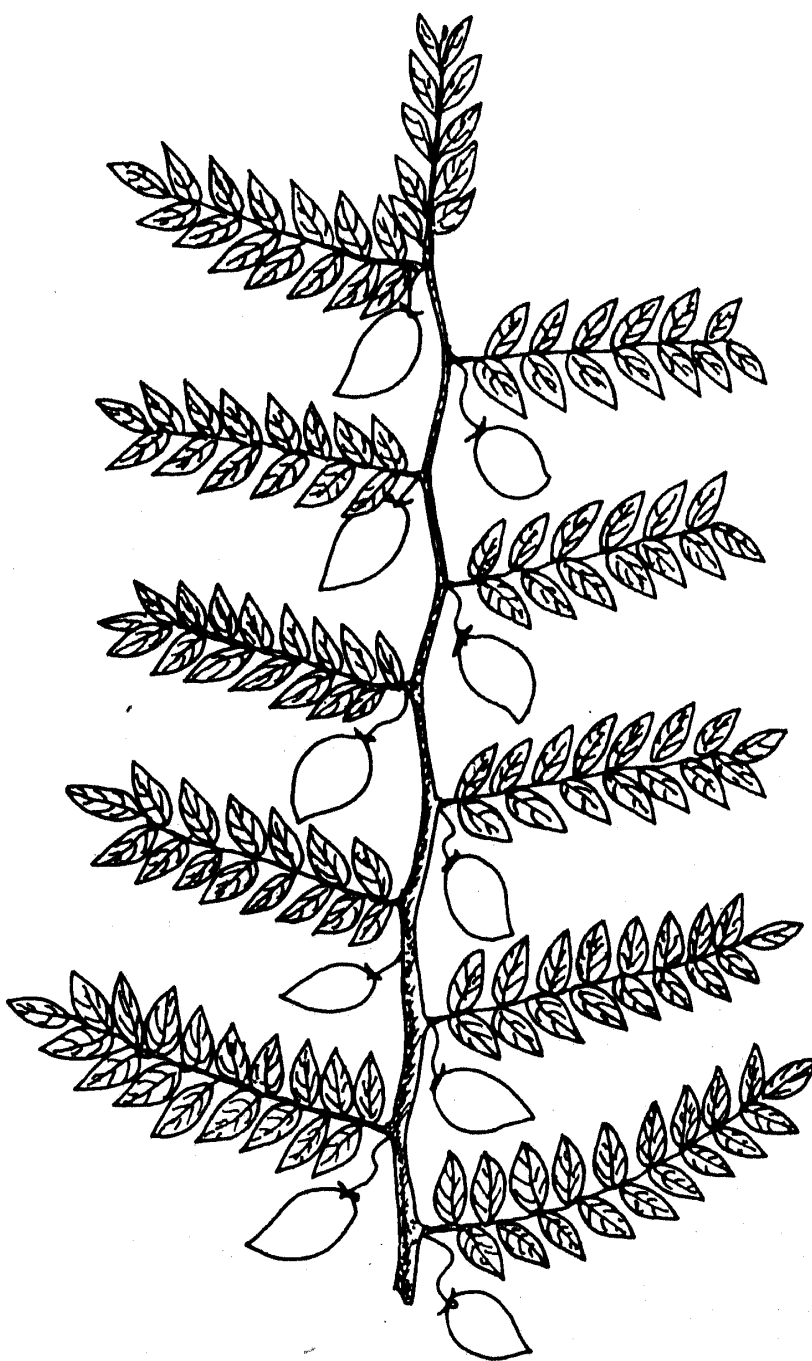


Figure 1. Apical portion of the main axis in chick pea. Top 8 nodes were used in the present work.

gels in 7% acetic acid, gel scanning was done at 620 nm by Gilson spectrophotometer. Freshly harvested leaf tissue was extracted in chilled 80% acetone and absorbance was read at 663 and 645 nm using spectrophotometer for determining total chlorophyll content (Arnon 1949).

3. Results

The dry matter accumulation in pods in the phase from flowering to young pod stage (phase I) was 35% of the total pod weight at harvest (table 1). The individual contribution of podwall and seed weight was 62 and 38%, respectively. In phase II (young pod to mature pod stage), the dry matter accumulation in pods was 65% of the total pod weight out of which the individual contribution of podwall and seeds was 27 and 73% respectively. Of the total dry weight at harvest, the podwall and seeds accumulated 81 and 18%, respectively in phase I and 19 and 82%, respectively, in phase II.

Leaf net photosynthesis rate, subsequent to the flowering, declined appreciably (table 2). The extent of its loss was higher in control plants as compared to deflowered and depodded plants. RuBP carboxylase activity also showed a continuous decline with the initiation of pod growth but unlike net photosynthesis rate, the extent of loss

Table 1. Dry matter accumulation in pod, podwall and seeds in chick pea.

Stage	Component	Dry matter, mg ±SE
Young pod	Podwall	32.8 ± 1.8
	Seeds	20.0 ± 0.58
	Pods	52.8 ± 1.51
Mature pod	Podwall	40.4 ± 2.55
	Seeds	109.6 ± 6.39
	Pods	150.0 ± 8.33

Values are given on per pod basis. SE represents standard error.

Table 2. Changes in net photosynthesis rate in leaves of control, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	Net photosynthesis rate ± SE (mg CO ₂ dm ⁻² h ⁻¹)
Flowering		31.3 ± 2.9
Young pod	Control	10.7 ± 1.0
	Deflowered	24.5 ± 0.6
Mature pod	Control	7.1 ± 1.6
	Deflowered	19.6 ± 2.6
	Depodded	8.3 ± 1.4

SE represents standard error.

Table 3. Changes in the RuBP carboxylase activity in leaves of control, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	RuBP carboxylase activity \pm SE ($\mu\text{mol CO}_2 \text{ g fwt}^{-1} \text{ h}^{-1}$)
Flowering		339 \pm 12
Young pod	Control	275 \pm 2.0
	Deflowered	295 \pm 11.0
Mature pod	Control	51.6 \pm 3.7
	Deflowered	68.6 \pm 0.7
	Depodded	61.9 \pm 0.3

SE represents standard error.

in RuBP carboxylase activity was higher in phase II as compared to phase I (table 3). The leaves from Df plants showed 7 and 33% higher RuBP carboxylase activity compared to control at young and mature pod stages, respectively. As against C, leaves from depodded plants at mature pod stage showed 20% higher enzyme activity.

The densitometer tracings of the electrophoretic pattern of the soluble proteins are given in figure 2. The initial broad peak was taken as corresponding to RuBP carboxylase, based on the R_f value obtained with purified RuBP carboxylase protein in a separate run under identical conditions. By the end of phase I, the loss in peak area was 11 and 3% in C and Df treatments respectively as compared to flowering. The corresponding loss at mature pod stage was 61, 34 and 54% in C, Df and Dp treatments, respectively. Total chlorophyll content, like RuBP carboxylase activity and RuBP carboxylase protein, was reduced more in phase II compared to phase I (table 4). At young pod stage, its amount in C and Df leaves was same. However, Df leaves showed 61% more chlorophyll content than control at mature pod stage. The leaves from control plants were shed appreciably earlier than leaves from Df and Dp plants.

4. Discussion

It has often been observed in grain legumes such as pigeon pea, chick pea, mung bean and many other species that the branches or leaves having no pods in their axil remain green, while on the same plant, the leaves subtending pods turn yellow and become senescent. In chick pea, data presented in tables 2, 3 and 4 reflect that the leaves, from the axil of which either the flowers or pods were removed, exhibited relatively delayed senescence as judged by the flowering-onwards loss of net photosynthesis rate, RuBP carboxylase activity, RuBP carboxylase protein and total chlorophyll content. Taking chlorophyll level as a marker, similar conclusions have earlier been reported for soya beans (Leopold *et al* 1959; Lindoo and Nooden 1976). However, the mechanism through which the developing reproductive structures induce early senescence of the leaf which subtends, remains largely unknown. The increased remobilization of assimilates and the active transport of some hormonal signal are the

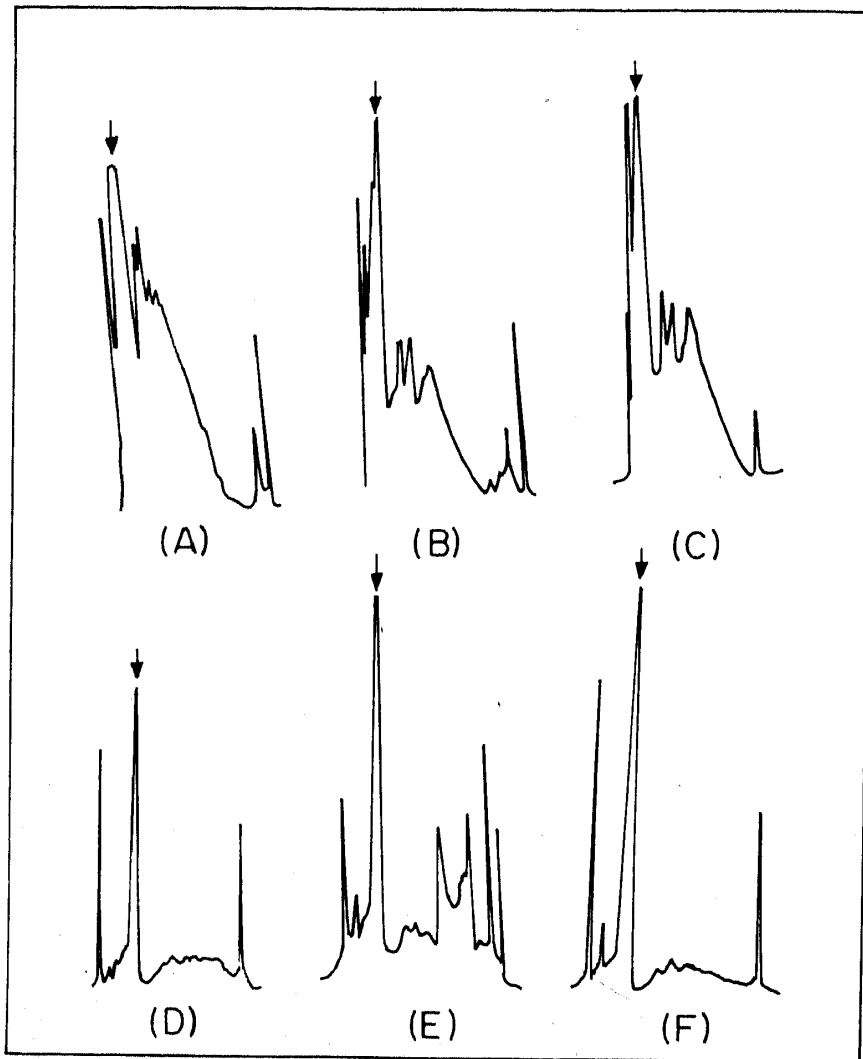


Figure 2. Densitometer traces of soluble proteins of chick pea leaves separated by discontinuous polyacrylamide gel electrophoresis. Direction of migration from left to right in each case. Arrows represent the band of RuBP carboxylase protein. **A.** Flowering stage. **B.** Young pod stage, control. **C.** Young pod stage, deflowered treatment. **D.** Mature pod stage; control. **E.** Mature pod stage, deflowered treatment. **F.** Mature pod stage, depodded treatment.

two major theories which have so far been propounded in this respect (Sesay and

ation in developing pods in chick pea revealed the initial stages and seeds at the later stages. A due to its own photosynthetic magnitude (Khanna-Chopra s, particularly for nitrogen, s due to failure in supply from phase of pod growth. The net ly phase of pod development nutrient drain, some other at role in triggering the loss of

Table 4. Changes in the total chlorophyll content in leaves of C, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	Total chlorophyll content \pm SE (mg g fwt ⁻¹)
Flowering		2.15 \pm 0.07
Young pod	Control	1.80 \pm 0.12
	Deflowered	1.83 \pm 0.10
Mature pod	Control	0.95 \pm 0.06
	Deflowered	1.53 \pm 0.11
	Depodded	1.09 \pm 0.02

SE represents standard error.

net photosynthesis rate in chick pea leaves. The major decline in RuBP carboxylase activity and RuBP carboxylase protein was associated with the active phase of seed growth; it is generally believed that amino-nitrogen released due to the hydrolysis of RuBP carboxylase protein is preferentially transported to developing grains (Williams and Kennedy 1978; Friedrich and Huffaker 1980).

Loss of chlorophyll content was more close to the diminution of RuBP carboxylase activity than that of net photosynthesis rate throughout senescence. The rate of net photosynthesis and RuBP carboxylase activity (*in vitro*) contributed in a differential manner towards decline of photosynthesis during leaf senescence in chick pea. To explain similar findings in barley, Friedrich and Huffakar (1980) proposed that factors such as *in vivo* regulation and stomatal aperture besides *in vitro* RuBP carboxylase activity are equally important in controlling photosynthetic rate.

The evidence in this paper suggests that the flowering-onwards loss in net photosynthesis rate on the one hand and RuBP carboxylase activity and RuBP carboxylase protein, on the other hand, in chick pea are non-parallel. Whereas the change in RuBP carboxylase protein characteristics was associated with the stage of rapid seedfill, no such association was marked for the loss in net photosynthesis rate. Thus both nutrient remobilization and hormonal action are probably involved in monocarpic senescence in chick pea. Photosynthetic assimilation has been shown to be a limiting factor in productivity of grain legumes (Hardy *et al* 1978). A closer look into the role of developing reproductive sink in triggering the decline of photosynthetic components of the subtending leaves may help in evolving strategies for further increasing yield in these crops.

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