Translocation of ³²P from leaves to seeds of sunflower and associated biochemical changes in young and mature leaves during seed-filling

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Abstract

Transport of ³²P from mature leaves was studied at stage I (ray florets opening), stage II (50% florets opening) and stage III (central disc florets opening) of the capitulum in order to ascertain the capacity of developing seeds to draw metabolites. Experiments showed that when feeding was done at stage I separately in the 6th, 7th and 8th leaf from the apex, accumulation of ³²P was maximum in the peripheral seeds of the corresponding zone of the fed leaf. Also at stage II was ³²P accumulation high in the peripheral zone along with high accumulation in the inner zone. But at stage III and IV (seven days after central disc florets opening) when accumulation declined considerably in the peripheral zone, very little accumulation of ³²P was noted in the central zone of the capitulum. During maximum sink demand i.e. at stage II, the soluble cabohydrate level increased both in apically borne young and mature leaves but declined thereafter only in mature leaves. In both young and mature leaves the chlorophyll level, however, did not show any decline even at stage IV. It was further observed that dry matter retention capacity declined only in young leaves from stage II onwards. In young leaves, catalase activity was low from stage II whereas in mature leaves it was low at stage II followed by a transient sharp increase at stage III.

Key words: sunflower, ³²P translocation, carbohydrate, dry weight, chlorophyll, catalase

INTRODUCTION

It is well known, that the major problem with the sunflower is poor seed-filling and, in fact, many seeds remain only partially filled and the seeds of the central zone of the capitulum remain almost unfilled.

In crop plants there are several limitations regarding good seed setting and development (L u c i a n o et al. 1965, R a w s o n and E v a n s 1970, E v a n s et al. 1972, K h a n n a 1972, P a t i l et al. 1976). Such limitations are ascribed either to sink or source and the translocating system generaly posses no barrier in this regard (G e i g e r et al. 1969, E v e n s et al. 1970). With fertilization and consequent establishment of sink demand, some biochemical changes occur within the source and then metabolites are depleted towards the sink. Sources, such as leaves, change their function to exporter from importer when they have completed 40-50% expansion (G i a q u i n t a 1978). Again, with concomitant establishment of sink demand, photosynthetic activity of source leaves increases as is evident from the studies of several workers (H a n s e n 1970, T h o r n e and K o l l e r 1974, P a t t e r s o n et al. 1980).

In sunflower most of the workers (J o h n s o n 1972, S r i v a s t a v a et al. 1976) reported that lower leaves (6-8th) of the upper mature set of leaves were more contributory and uppermost apically borne expanding leaves were almost noncontributory. P r a s a d et al. (1977) found that mobilization of ^{14}C-metabolites into the head of the sunflower increased by the application of growth regulators. From experiments with ^{14}C, M c W i l l i a m et al. (1974) showed that the large and photosynthetically active leaves in the upper part provided most of the carbon assimilated to the inflorescence during the seed-filling period. So translocation of metabolites depends on both source and sink capacity simultaneously.

Considering the aforesaid views, in the present investigation an attempt has been made to analyse the ability of seed tissues of different zones of the capitulum to draw metabolites from source leaves at different stages of seed-filling. Again, the metabolic status of leaves was also diagnosed during the process of seed-filling both in young and mature leaves. Such an analysis permitted the screenning out of the role of upper leaves in the process of loading. The analyses also appeared to reveal some of the major causes of poor seed-setting, especially in the central zone of the capitulum.

MATERIAL AND METHODS

Certified seeds of sunflower (EC 68414) were collected from Crop Research Farm, Burdwan University. Experiments were performed with plants which were grown under field conditions. Seeds were sown at 40 cm × 30 cm spacing.

Translocation of metabolites from source to sink was studied by feeding ^{32}P during seed-filling stages. Such feeding was made to the 6th, 7th and 8th leaves from the apex of the sunflower plant. J o h n s o n

(1972) from his experiments conclusively determined that the upper leaves of the middle set of leaves were more contributory towards seed-filling. His findings were accepted and in this investigation the radioactivity chemical was fed only to such leaves in order to observe their ability to contribute the feed chemicals to the seeds. Feeding was made to the leaves at different stages of seed-filling of the plant i.e. at 1) ray florets-opening (stage I), 2) 50% floret-opening (stage II), 3) central disc floret-opening (stage III) and 4) seven days after central disc floret-opening (stage IV). After 48 hrs of feeding, the heads were detached. To study the accumulation of ³²P in the capitulum, it was divided into five zones as the phyllotaxy was pentastichous. Materials of different portions were dried under an infra-red lamp and the radioactivity was measured with the help of a G. M. Counter following standardized procedure (N a k a y a m a and V a n b a v e l 1963). Three uniformly growing plants were taken as replicates for each treatment. The mean value of the count was considered.

In performing biochemical analyses, leaf samples were collected from large and mature leaves (6-8th) as well as from small and young apically borne leaves (1st-3rd) which were proximal to capitulum.

The following parameters were considered in leaves at different stages of seed-filling: 1) leaf area, 2) dry weight, 3) chlorophyll, 4) soluble carbohydrates and 5) catalase activity.

The area of each leaf was measured with the help of graph paper (mm), dry weight of the leaves was determined following the method of L o o m i s and S h u l l (1937). The chlorophyll level was estimated by A r n o n's method (1949), soluble carbohydrate was estimated following the method described by D w i v e d i et al. (1979), catalase was extracted from the sample following the method of B i s w a s and C h o u d h u r i (1978). Catalase activity was estimated according to the method of S n e l l and S n e l l (1971). Five replicates were done for each case. During enzyme assay, zero time was taken as blank and the activity was expressed as the decrease in optical density per unit time per unit weight of the tissue (Δ OD·time^{-1}·wt.$^{-1}$).

RESULTS AND DISCUSSION

In the present investigation, ³²P was applied in some of the large and photosyntheticaly active leaves at definite intervals from the inception of anthesis (Fig. 1). In the sunflower there are many hollow seeds for which various reasons are assigned (L u c i a n o et al. 1965, K h a n n a 1972). The results showed that the central zone has the least drawing capacity for metabolites even after sufficient days of anthesis. It was further observed that in the seeds of the outer zone of the capitu-

lum corresponding to the fed leaves (6-8th), ^{32}P accumulation took place to a significant extend even up to 10 days of inception of anthesis and the level declined thereafter.
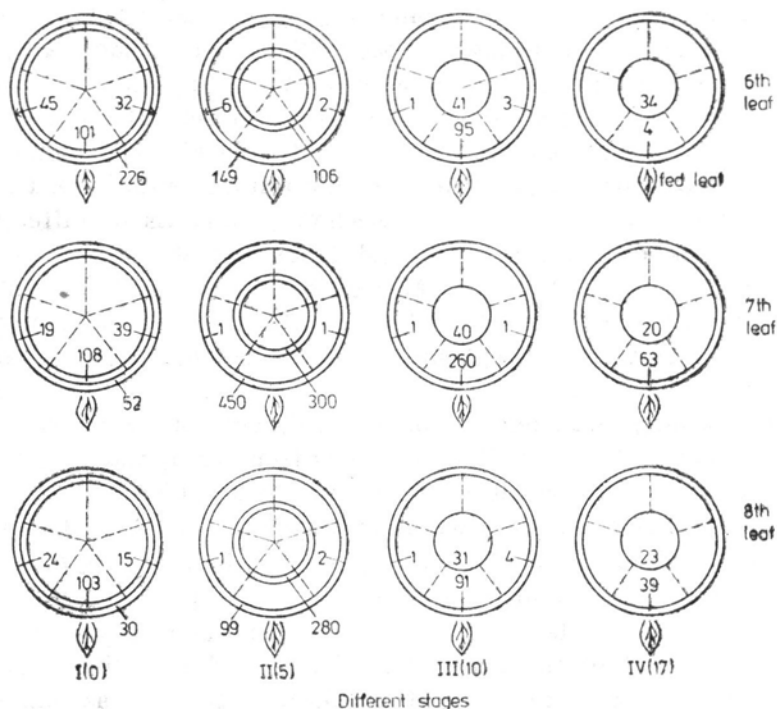

Fig. 1. Distribution of ^{32}P (cpm·mg^{-1} dry wt.) in different positions of sunflower head from 6th, 7th and 8th leaves from apex at different stages of seed-filling. In the figure, the head was divided into five zones because of pantastichous phyllotaxy. The distribution of ^{32}P in the corresponding zone of the feeding leaf and also in two adjacent zones were given. Outer rim represents the two outermost seed rows. Inner rim of each stage represents the positions where count was taken. Innermost circle represents the central zone

In considering the cause of hollowness of the seeds of the central zone, the question of insufficient supply from the source did not arise because ^{32}P was fed prior to each sampling. Thus it appeared that the seeds of the central zone failed to accumulate ^{32}P because of their unsuccessful development and in this regard the deficiency of an endogenous hormone might be one of the major causes (Seth and Wareing 1964, 1967, Wagner 1974).

Though the question of mutual competition for drawing metabolites by the seeds of the capitulum was often suggested (Luciano et al. 1965, Patil et al. 1976), the present observations did not speak in its favour. ^{32}P accumulation was significantly low in the outer zone after 15-18 days of anthesis. Concomitantly, accumulation of ^{32}P in the central

zone was not increased. Therefore, in the present study, it was observed that neither source limitation nor mutual competition for nutrients restricted maximum seed setting in the sunflower. The limitation was associated with the seeds themselves which probably failed to grow successfully during the course of development.

With the establishment of sink demand, biochemical changes occurred within the source leaves for translocation of metabolites to the sink (Thrower 1962, Patterson et al. 1980). It was reported that leaves of dicot take up the function of an exporter after 40-50% expansion (Giaquinta 1978). The uppermost 1st-3rd leaves of the sunflower retained more than 50% expansion potential during opening of ray florets i.e. at stage (Table 1). On the other hand, mature leaves (6-8th) exceeded more than 60% expansion at that stage. The total photosynthetic area of the 1st-3rd leaves was, however, far below the mature leaves when their expansion completed. In fact, the young leaves were still developing during the reproductive phase of the plant when mature leaves (6-8th) were almostly fully developed. Analyses revealed that dry

Table 1

Expansion percentage of leaves of sunflower plant at different stages of seed-filling

Leaf no. from apex	Leaf expansion (%) at different seed-filling stages			
	I	II	III	IV
1st	44 (3.96)*	54 (4.86)	90 (8.1)	100 (9)
2nd	43 (6.45)	64 (9.6)	92 (13.8)	100 (15)
3rd	45 (13.5)	69 (20.7)	88 (26.4)	100 (30)
6th	64 (42.24)	73 (48.18)	98 (64.68)	100 (66)
7th	70 (61.60)	72 (63.36)	100 (88)	100 (88)
8th	76 (63.46)	81 (67.63)	100 (83.5)	100 (83.5)

* Figures within parentheses indicate leaf area (cm^2).

Table 2

Changes in dry weight levels (mg·g^{-1} dry wt.) in young and mature leaves of sunflower during different stages of seed-filling

Stages of seed filling	Young leaf			Mature leaf		
	1st	2nd	3rd	6th	7th	8th
I (0)*	220 ab**	241 a	241 b	252 a	254 a	254 a
II (5)	238 a	248 a	260 a	252 a	250 a	260 a
III (10)	202 bc	216 b	238 b	252 a	252 a	252 a
IV (17)	186 c	202 b	214 c	236 a	248 a	248 a

* Figures within parentheses indicate days after ray florets opening.
** Within a column the same letters are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

matter retention capacity of young leaves after being increased in stage II (50% anthesis), declined progressively but this remained almost unchanged in mature leaves (Table 2). Such an observation simply on the basis of dry matter retention capacity, showed that though the young leaves attained their maturity during a later phase, they depleted their metabolites to the developing sink more efficiently. The cause of this may be assigned to their proximal association with the sink i.e., capitulum.

In both types of leaves, the soluble carbohydrate level increased from commencement of anthesis stage i.e. stage I to 50% anthesis i.e. stage II (Table 3). This suggested that irrespective of their expansion potentiality,

Table 3

Changes in soluble carbohydrate levels (mg $\cdot$g^{-1} dry wt.) in young and mature leaves of sunflower during different stages of seed-filling

Stages of seed-filling	Young leaf			Mature leaf		
	1st	2nd	3rd	6th	7th	8th
I (0)*	36 c**	22 c	37 c	34 b	52 b	50 b
II (5)	46 b	41 b	62 a	61 a	64 a	67 a
III (10)	60 a	59 a	56 ab	40 b	37 c	25 c
IV (17)	60 a	54 a	49 b	22 c	27 d	21 c

* Figures within parenthesis indicate days after ray florets opening.
** Within a column the same letters are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

the carbohydrate synthetic ability of these leaves increased when the sink demand reached its maximum peak. Increase of photosynthetic activity during the seed-filling stage was noted in soybean (D o r n h o f f and S h i b l e s 1970, G h o r a s h y et al. 1971). In the apple tree, leaves subtending fruit may have a photosynthetic rate more than 50% higher than the corresponding leaves without fruit (K o z a r y a n et al. 1965, H a n s e n 1970). During active sink demand, the rate of photosynthesis increased which was evident by the increase in the activity of RuPBcase (T h o r n e and K o l l e r 1974, P a t t e r s o n et al. 1980). Therefore, the rate of photosynthesis was controlled by the demand for the assimilate, and photosynthesis, storage and supply may apper to be balanced pheonmena. The results in the present investigation showed that the higher level of soluble carbohydrate was maintained only in the young leaves for a long duration i.e. up to stage IV. Both mature and young leaves showed no remarkable decrease in the chlorophyll level even up to stage IV (Table 4). The higher level of soluble carbohydrate in the young leaves in comparison with mature leaves during the later period of seed--filling may indicate higher photosynthetic activity in the young leaves.

Table 4

Changes in chlorophyll levels (mg·g⁻¹ dry wt.) in young and mature leaves of sunflower during different stages of seed-filling

Stages of seed-filling	Young leaf			Mature leaf		
	1st	2nd	3rd	6th	7th	8th
I (0)*	0.92 a**	1.04 a	0.81 a	1.36 a	1.39 a	1.50 a
II (5)	0.66 ab	0.98 a	1.04 a	1.01 a	1.11 b	1.52 a
III (10)	0.52 b	0.84 a	0.92 a	1.27 a	1.10 b	1.13 b
IV (17)	0.46 b	0.92 a	0.90 a	1.10 a	1.10 b	1.10 b

* Figures within parenthesis indicate days after ray florets opening.

** Within a column the same let'ers are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

Such observations from the present analyses lead one to suggest that though the dry matter retention capacity of the young leaves was found to be lower during the later phase of seed-filling in comparison to mature leaves, their synthetic ability and probably the supplying potential of soluble substrate to the sink were more efficient. Observations thus showed that because of residual sink demand in the centrally located seeds of the capitulum during the later period of seed-filling, synthetic ability persisted only in young leaves for a longer duration.
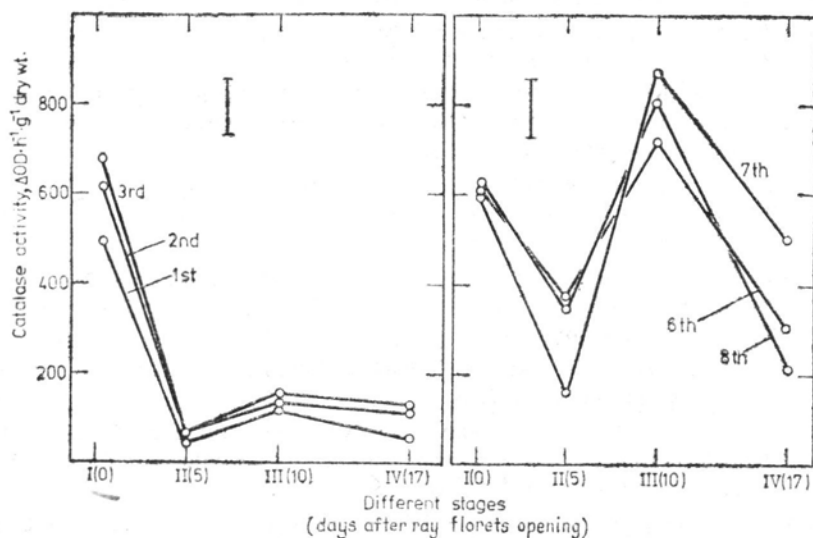

Fig. 2. Changes in catalase activities in young and mature leaves of sunflower during different stages of seed-filling. Vertical bars represent critical difference (CD) at P = 0.05

In stage II i.e. at 50% anthesis, sink demand was maximum which has been established from ³²P mobilization experiments. At this stage, augmented metabolism was noted both in young and mature leaves.

In the present investigation, decreased oxidative enzyme activity of catalase was observed at this stage (Fig. 2). This suggested that the rate of degradation of available substrates during this stage was minimal and as such oxidative enzyme production was limiting. In mature leaves activity of catalase increased sharply at stage III followed by a decline at stage IV, but the young leaves maintained almost steady but low activity throughout the period of observation from stage II onwards. Such data of decreased catalase activity in young leaves from stage II onwards were suggestive of the fact that in such leaves oxidative processes were at low ebb throughout the period of seed-filling.

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Przemieszczanie ³²P z liści do nasion słonecznika i zmiany biochemiczne w młodych i dojrzałych liściach w okresie wypełniania się nasion

Streszczenie

Badano transport ³²P z dojrzałych liści w stadium I (kwitnienie kwiatków obwodowych), II (50⁰/₀ rozchylonych kwiatków) i III (kwitnienie kwiatków tarczy środkowej) głowy słonecznika aby ocenić zdolności rozwijających się nasion do pobierania metabolitów. Badania wykazały, że gdy podawano ³²P w stadium I oddzielnie na liście 6, 7 i 8 od szczytu, akumulacja tego izotopu była większa w obwodowych nasionach w strefie odpowiadającej liściowi któremu podawano ³²P. Również w stadium II, akumulacja ³²P była większa w strefie obwodowej wraz z dużą akumulacją w rejonie środkowym. Jednakże, w stadiach III i IV (7 dni po zakwitaniu kwiatków tarczy środkowej), kiedy akumulacja w strefie obwodo-

wej znacznie zmalała, zanotowano bardzo małą akumulację ^{32}P w środkowym rejonie tarczy słonecznika. Podczas okresu największego zapotrzebowania na metabolity, tj. w studium II, poziom rozpuszczalnych węglowodanów znacznie wzrósł zarówno w szczytowych młodych jak i dojrzałych liściach, zmalał jednak później tylko w liściach dojrzałych. Jednakże, zarówno w młodych jak i dojrzałych liściach, poziom chlorofilu nie wykazywał spadku nawet w stadium IV. Zaobserwowano że zdolność do utrzymywania suchej masy malała tylko w młodych liściach od stadium II. W młodych liściach aktywność katalazy była mała począwszy od stadium II podczas gdy w liściach dojrzałych była mała w stadium I a następnie miał miejsce przejściowy wzrost w stadium III.