Vol. 85, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1978 Pages 921-926

# HIGHER THAN LEAF HILL ACTIVITY OF COTYLEDONARY CHLOROPLASTS FROM DEVELOPING LEGUME SEEDS

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Received August 11, 1978

Abstract.- The chlorophyll composition and Hill activity of the leaf, developing seed parts and pod have been studied in three species of legumes, *Lathyrus latifolius*, *Pisum sativum* and *Vicia faba*. The studies indicate that in all the species, the level of chlorophyll, on mg/g fresh weight basis, is maximum in the leaf. However, Hill activity studies show that the cotyledonary chloroplasts in all the cases have a higher Hill activity than the leaf chloroplasts. Thus, the Hill activities of the cotyledonary chloroplasts are 340% of the leaf chloroplasts in *Lathyrus latifolius*, 144% of the leaf chloroplasts in *Pisum sativum* and 200% of the leaf chloroplasts in *Vicia faba*.

## Introduction.-

The occurrence of chlorophylls in the inner parts of many angiospermous seeds is well established[1]. Although, biochemical changes during seed development have been studied and reviewed[2,3], little attention has been paid to the role of chlorophylls in such deep seated parts as the cotyledons in developing seeds. It was, therefore, of interest to undertake a comparative study of the chlorophyll composition and Hill activity of the seed parts, pods and leaves of certain legumes. The main aim was to derive an idea of the photosynthetic capacity of the cotyledonary chloroplasts relative to those of the leaves i.e. to check whether the chlorophylls in the deep seated cotyledonary chloroplasts, receiving much reduced light, are non-functional or they have some functional significance.

0006-291X/78/0853-0921$01.00/0

## Materials and Methods.-

Materials: Seeds of *Lathyrus latifolius*, *Pisum sativum* T163 and *Vicia faba*, harvested from plants grown in the Meerut University experimental plots, were used for the experiments. The plants from such seeds were grown in the experimental plots and the flowers tagged on their first day of opening (anthesis). Pods were collected at 25 days after anthesis and the pods and the seed parts i.e. the seed coat and the cotyledon were dissected apart for analysis. For proper comparison, the leaves subtending such pods were also collected. One g samples of pod, seed coat, cotyledons and leaves subtending the selected pods were analysed for chlorophyll content, composition, chloroplast isolation and subsequent determination of Hill activity and total chlorophyll content of the isolated chloroplasts.

Chlorophyll Analysis: The tissue was homogenised in 80% acetone, filtered, the pigments transferred to diethyl ether, the ether extract dried over anhydrous sodium sulphite and made to 10 ml with ether. The absorbance of the extract was read at 644 and 662 nm in a Bausch and Lomb Spectronic-20 Spectrophotometer and mg chlorophyll-a (Chl.a) and chlorophyll-b (Chl.b) contents determined[4,5,6].

Hill Activity: Chloroplast isolation and the determination of Hill activity and total chlorophyll estimation, of the chloroplast suspensions, were done according to the method given in a previous paper[7]. Briefly, for chloroplast isolation known amount of fresh tissue was homogenised in a grinding medium (0.35 M NaCl, 0.1 M Tris-HCl, pH 8.0), filtered, centrifuged first at 100xg for 1 min. and the resulting supernatant at 1000xg for 5 min. The chloroplast pellet was suspended in a suspending medium (0.175 M NaCl, 0.1 M $K_2HPO_4$-$KH_2PO_4$, pH 6.5) to a final volume of 1 ml, after washing by centrifugation in the same medium. The whole process was carried out in cold. Half of this suspension (A) was used for Hill activity and the residual 0.5 ml for chlorophyll estimation. For Hill activity, 0.5 ml of suspension A was made to 25 ml in the suspending medium (B). 5 ml aliquots of B was mixed rapidly with 0.1 ml of $3.5 \times 10^{-3}$ M 2, 6 Dichlorophenolindophenol (DCPIP), exposed to 560 Lux light (through a water jacket from two 100 W tungsten bulbs kept 20 cm away) for 5 min and the OD at 620 nm read against control blanks kept in dark. For chlorophyll estimation in an equivalent volume of chloroplast suspension, 0.1 ml aliquots of suspension A were mixed thoroughly with 4.5 ml acetone, centrifuged and the OD of the supernatant read at 650 nm to calculate the total chlorophyll[8]. Hill activity has been expressed as µM DCPIP reduced/mg chlorophyll/ minute.

## Results and Discussion.-

The results given in Table 1 show in each case that the coty-

Table -1 : Chlorophyll composition and Hill activity of the leaf, seed parts and pod of *Lathyrus latifolius*, *Pisum sativum*, T.163 and *Vicia faba*, 25 days after anthesis.

| Organ | Chlorophyll Composition mg/gram fresh weight | | Ratio | Hill activity µM DCPIP reduced/ mg chlorophyll/ min. |
|---|---|---|---|---|
| | Chl.a. | Chl.b. | Chl.a:Chl.b. | |
| | | *Lathyrus latifolius* | | |
| Leaf | 0.830 | 0.600 | 1.00:0.72 | 0.016 |
| Cotyledon | 0.100 | 0.070 | 1.00:0.65 | 0.055 |
| Seed Coat | 0.060 | 0.030 | 1.00:0.53 | 0.018 |
| Pod | 0.120 | 0.070 | 1.00:0.55 | 0.023 |
| | | *Pisum sativum* | | |
| Leaf | 0.830 | 0.460 | 1.00:0.56 | 0.045 |
| Cotyledon | 0.060 | 0.040 | 1.00:0.63 | 0.065 |
| Seed Coat | 0.009 | 0.008 | 1.00:0.89 | 0.046 |
| Pod | 0.043 | 0.026 | 1.00:0.61 | 0.050 |
| | | *Vicia faba* | | |
| Leaf | 0.917 | 0.599 | 1.00:0.65 | 0.030 |
| Cotyledon | 0.065 | 0.051 | 1.00:0.79 | 0.060 |
| Seed Coat | 0.021 | 0.016 | 1.00:0.79 | 0,030 |
| Pod | 0.092 | 0.056 | 1.00:0.61 | 0.026 |

ledonary chloroplasts have higher Hill activity than the leaf chloroplasts.

The data indicate that in all the three cases studied, the level of chlorophylls is maximum in leaves and the seed parts and pod contain much lower amounts of chlorophyll. The levels of individual pigments in the leaves are ca. 10 to 20 times higher than those of the seed parts and pod. As regards the pigment composition, in *Lathyrus latifolius* the Chl. a: Chl. b ratio of the cotyledon more nearly approximates that of leaf. The pigment composition of the seed coat and pod are similar but both differ from the leaf in having lower amounts of Chl.b relative to Chl.a. In *Pisum sativum* the chlorophyll compositions of the leaf, cotyledon and pod are about similar and the seed coat differs in having relatively higher amount of Chl. b relative to Chl. a. In _Vicia faba_, the table shows that the chlorophyll composition of the pod is similar to that of the leaf while those of the cotyledon and the seed coat differ in having higher amounts of Chl. b relative to Chl.a.

The most interesting feature is with regard to the Hill activity of the chloroplasts from the pod and seed parts as compared to the activity of the leaf chloroplasts. The data indicate the singular feature that the Hill activity of the cotyledonary chloroplasts is higher than that of the chloroplasts from leaves in all the cases studied. The ratio of the Hill activities of leaf : pod : seed coat : cotyledon in _Lathyrus latifolius_ is 1.0 : 1.4 : 1.1 : 3.4. The Hill activity of the cotyledonary chloroplasts in this plant is, thus, 340% of the leaf chloroplasts. In _Pisum sativum_ at 25 days after anthesis the Hill activity of the leaf chloroplasts is 0.045 μM DCPIP (2, 6-Dichlorophynolindophenol) converted/ mg chlorophyll/min. The activities of the pod and seed coat chloroplasts are about the same. However, the activity of the cotyledonary chloroplasts is ca, 40% more than that of the leaf chloroplasts. In _Vicia faba_ a similar pattern is observed. At 25 days after anthesis the Hill activity of the pod and the seed coat chloroplasts is almost same as those of the leaf chloroplasts i.e. ca. 0.03 μM DCPIP converted/mg chlorophyll/min. The cotyledonary chloroplasts again show 100% more activity than the leaf chloroplasts.

Thus, in all the three cases observed, the cotyledonary chloroplasts, although occurring in a deep seated region, show higher Hill activity than the leaf chloroplasts. It is worthy of note that analyses had shown that at 25 DAA the subtending leaf had highest Hill activity in comparison to the earlier (15 days after anthesis) and later (35 days after anthesis) stages of seed development.

The demonstration of Hill activity in the pod and seed coat, during the stages of development studied, indicates the possibility of their having some self sufficiency with regard to growth, as also observed in developing red kidney bean pods[9]. The possibility of these parts contributing partly some raw materials to the developing cotyledon can not also be ruled out. The high Hill activity of the cotyledonary chloroplasts *in vitro* indicates, although does not prove, their being photosynthetically active during seed development *in vivo*. The cotyledon thus, partly may be synthesizing its own reserves. However, it is worthy of note that the light reaching these chloroplasts through the pod and the seed coat etc., ought to be of much reduced intensity and this raises several queries as to the *in vivo* photosynthetic activity of the deep seated cotyledonary chloroplasts. Further work seems imperative to find answers for these and related problems.

Thanks are due to Dr. Y.S. Murty, Prof. & Head of the Department of Botany for providing facilities and constant encouragement and to the Indian Council of Agricultural Research for financial support.

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