IMPAIRMENT OF CHLOROPLAST DEVELOPMENT AND SINK STRENGTH BY BLOCKADE OF LIGHT IN CHLOROEMBRYOS OF *CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.

P. Kaladharan* and M. Vivekanandan, Department of Botany, Bharathidasan University, Tiruchirapalli 620 024, INDIA

*Scientist S-1, Central Marine Fisheries Research Institute, Cochin 31, INDIA

ABSTRACT

In many members of Fabaceae synthesis of chloroplast pigments takes place even when the embryo is deep inside the fruit. Blockade of light totally etiolated the embryos of developing *Cyamopsis* fruits upto 16-18 days after anthesis (DAA), whereas the pigments of the mature embryos, when shaded, were not significantly affected. Upon illumination, resynthesis of green pigments by etiolated embryos occurred both *in vivo* and *in vitro* more significantly during the early stages of growth of the embryos (upto 18 DAA). Shading of developing fruits at different growth stages of embryos, reduced the growth of sink. The results are discussed in relation to the importance of light on embryo greening and dry matter production.

1. INTRODUCTION

'Chloroembryos' has been the subject of much curiosity in recent times with regard to their location, development and function (Ryczkowski and Szewczyk, 1975; Yakovlev and Zhukova, 1980; Palanisamy and Vivekanandan, 1986; Kaladharan and Vivekanandan, 1989). The present report explains the vital role of photosynthetically active radiant energy on the formation of chloroplast pigments and sink strength in the developing chloroembryos of *Cyamopsis tetragonoloba* (L.) Taub, although they reside deep inside the endosperm, seed coat and pod wall.

2. MATERIALS AND METHODS

From the field-grown plants of *Cyamopsis tetragonoloba* Cv. Nowbagar, flowers were tagged on the day of anthesis. Developing pods were grouped into four stages based on the DAA, viz., stage I: embryos of 8-10 DAA; stage II: 16-18 DAA; stage III: 26-28 DAA; stage IV: 36-38 DAA. Developing intact pods of *Cyamopsis* were blocked from the radiant energy by shading half the pod with light proof dark

M. Baltscheffsky (ed.), Current Research in Photosynthesis, Vol. IV, 903–906.

polythene sheet in the form of sleeves, while other half was covered with sleeves of clear polythene sheet as control. The shaded pods were left intact continuously for 8 days sufficient to etiolate the embryos. Removal of sleeves from the etiolated pods was done to facilitate *in vivo* re-greening, and *in vitro* regreening was induced by exposing the detached etiolated embryos to continuous white light of 150 W m^{-2} for 12 h in Petri-plates lined with Whatman No.1 paper wetted with sterile water. Chloroplast pigments were determined by the method of Arnon (1949). Sink strength was expressed as mg dry matter per gram fresh weight of embryos.

3. RESULTS AND DISCUSSION

Formation of etiolated embryos as a result of shading of fruits clearly proves that certain amount of radiant energy passes through the fruit wall, seed coat and endosperm, and finally reaches the embryo confirming that chlorophyll synthesis in the embryos is by light-dependent pathway (Kaladharan and Vivekanandan, 1983). Further, blockade of light in developing fruits of *Cyamopsis* containing green embryos led to the breakdown of chlorophylls *a* and *b* in the embryos (Table 1) suggesting that a continuous supply of radiant energy is essential for maintenance of chloroplast pigments. Breakdown of chloroplast pigments in mature embryos (stage IV) is a slow process, whereas in younger ones (stages I-III) the process is faster, which may be attributed to faster turnover of pigments.

Table 1. Effect of shading of growing fruits of *Cyamopsis tetragonoloba* at various stages of growth on chlorophyll content of the embryos. (After 10 days of shading, the embryos were isolated from shaded and unshaded parts of the fruits and chlorophyll content was determined.)

Embryo		Chl. *a*	Chl. *b*	Carotenoids	Chl.*a*/*b*
		(μg gfw^{-1})			
Stage I	BS	205.54	106.27	104.86	1.93
	AS	4.62	3.55	15.94	1.30
Stage II	BS	534.80	252.01	211.40	2.12
	AS	12.70	9.12	12.09	1.39
Stage III	BS	411.57	271.11	210.17	1.52
	AS	31.04	25.37	19.04	1.22
Stage IV	BS	308.94	152.02	112.00	2.03
	AS	180.58	90.98	65.00	1.98

BS = Before Shading; AS = After Shading.

Removal of mask (dark sleeves) and reillumination of the fruits to natural light resulted in regreening of the etiolated embryos (Table 2) suggesting a normal light-induced development of chloroplasts from etioplasts as regulated by phytochrome (Lichtenthaler and Buschmann, 1978). Upon irradiation, more chloroplast pigments were formed in etiolated stage III than stage II embryos. It appears as though the embryos of *Cyamopsis* need to reach a certain stage of development to acquire optimum level of certain factor(s) in light before they can be induced to regreen upon illumination.

Table 2. Resynthesis of chloroplast pigments in *vivo* and *in vitro* by the embryos of *Cyamopsis tetragonoloba* shaded at different stages of growth

	Stage II		Stage III	
	Total chl.	Total carotenoids	Total chl.	Total carotenoids
	($\mu g\ gfw^{-1}$)			
Shaded embryos *In vivo*	8.17	15.94	21.82	12.09
5 days re-exposure *In vitro*	164.50	55.22	312.02	97.20
12 h after re-exposure	35.91	11.43	110.27	18.17

As shown in Fig. 1, shading young fruits of *Cyamopsis* just a day after anthesis for 8 days caused 65% reduction in drymatter of etiolated embryos than the green embryos of the same half of the fruit covered by clear polythene sheet. However, the etiolated part of fruitwall showed only 13% reduction. Similarly in stage II fruits reduction was 40% and 9% respectively in the embryos and fruitwall, and reduction of dry matter in the embryos of stages III and IV was 16% and 7% respectively without causing significant reduction in dry matter of fruit wall. Prevention of shading of fruit during early stages of development resulted in considerable reduction in dry matter of the embryos with only marginal reduction in fruitwalls. Similarly Hole and Scott (1981) observed that shading of pea fruits reduced yield in terms of number and size of seeds per fruit by 24% over the unshaded control.

Prevention of radiant energy in the black polythene-shaded part of the fruit probably resulted in limited synthesis of assimilates in the embryo, whereas the other half covered by clear polythene sheet under normal radiant energy synthesized their own assimilates by the self-sustained chloroembryos (Kaladharan and Vivekanandan, 1989). It

can be surmised from the present study that through the chloroembryos are deeply situated in the fruit, they are capable of utilising the diffused light for chloroplast pigments formation and synthesis of assimilates suggesting the possibility of a role for embryonal chlorophyll in photosynthesis as evidenced drastic dry matter reduction in the embryos of the shaded fruits, whereas that of fruit wall was least affected.

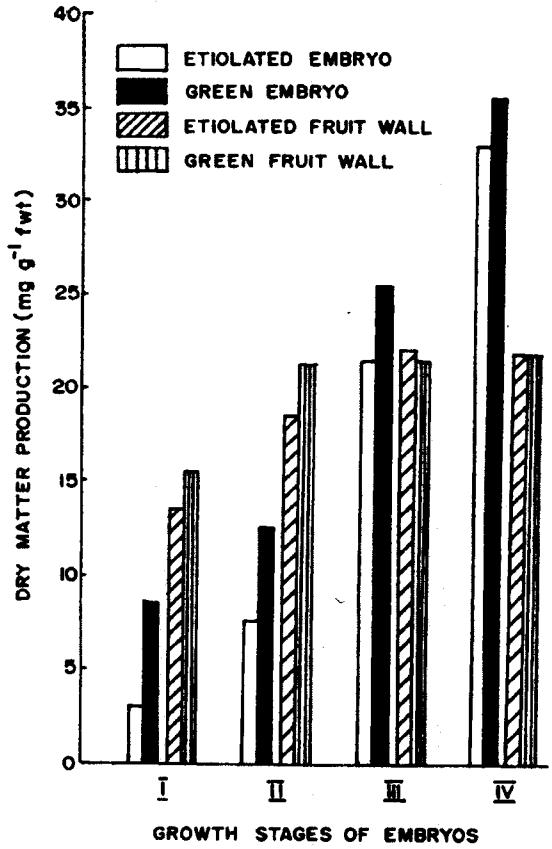

Fig. 1. Effect of shading of fruits of *Cyamopsis* on dry matter production of fruit wall and embryo

REFERENCES

1 Ryczkowski, M. and Szewczyk, E. (1975) Z. Planzenphysiol. 75, 175-180
2 Yakovlev, M. S. and Ahukdva, G. Ya. (1980) Bot. Notiser, 133, 323-336
3 Palanisamy, K. and Vivekanandan, M. (1986) Plant Physiol. 123, 395-399
4 Kaladharan, P. and Vivekanandan, M. (1989) Plant Physiol. (in press)
5 Arnon, D. I. (1949) Plant Physiol. 24, 1-15
6 Hole, C. C. and Scott, P. A. (1981) Ann. Bot. 48, 827-835