EFFECTS OF ILLUMINATION AND DARKNESS ON ANTHOCYANIN FORMATION IN BEET SEEDLINGS TREATED WITH OR WITHOUT N-PROPANOL

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ABSTRACT

Physiological studies on the biosynthesis of anthocyanin in beet seedlings have been carried out. A limited amount of anthocyanin occurs in dark-grown seedlings, when compared with that of illuminated ones. After four days there is a rapid synthesis of anthocyanin in both cotyledons and hypocotyls. The hypocotyl produces more pigment than the cotyledons. Seedlings treated with different concentrations of propanol, under continuous illumination and in the dark affects their content of anthocyanin pigment and total sugars differently. Increased propanol concentration inhibited pigment formation. Total sugars , also, decreased by increasing propanol concentration and the most effective concentration was 0.2%.

INTRODUCTION

It is well known that anthocyanin synthesis in a wide range of tissues is promoted by light. In apple skin (Siegelman, 1964) turnip seedlings (Siegelman and Hendricks, 1957) Celosia seedlings (Malaviya and Laloraya, 1966) and buckwheat hypocotyl (Troyer, 1964) anthocyanin synthesis is light dependent. These tissues fail to form anthocyanin if they are grown in darkness. The case of red cabbage (Pecket and Hathout, 1974) is somewhat different, since there is a significant dark synthesis of anthocyanin. Nevertheless, these authors also reported stimilatory effect of light on pigment synthesis. Oelmuller and Mohr (1985) found that anthocyanin formation in Milo seedlings occurs only in white light and blue, while red light and far-red light are totally ineffective.

The biosynthesis of anthocyanin pigment in intact seedlings or excised parts is recognised to be affected by many factors, particularly light and carbohydrate supply.

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In order to investigate the capacity of pigment formation in the cotyledons and hypocotyl, Grill and Vince (1964) severed the cotyledons from two-days old seedlings before exposure to 48hr light. Such severing increased the formation of anthocyanin in the cotyledons, the hypocotyl on the other hand, formed almost no anthocyanin when the cotyledons were removed.

It became clear to them that anthocyanin synthesis in the hypocotyl depends on the movement of substance(s) and that severing of the cotyledons increased the amount of anthocyanin in the cotyledons themselves. If light-dependent synthesis of anthocyanin in the hypocotyl was dependent on the amount of stored precursor moving from the cotyledons, it would be expected that the longer the cotyledons and hypocotyl remained in contact before severing, the greater the amount of anthocyanin would be formed in the hypocotyl (Gill and Vince, 1964). In other words, decrease in anthocyanin in the cotyledons would be accompanied by an increase in hypocotyl pigment. In fact they observed that the amount of anthocyanin synthesized in the hypocotyl was the same at all ages and almost negligible. They assumed that translocated storage material may be rapidly utilized by the growing hypocotyl in the dark and that none is available for anthocyanin synthesis when light is given. In the cotyledons the amount of pigment fell as the duration of illumination before severing was increased from 6 to 48hrs. Up to 6 hrs. of illumination before severing did not decrease the amount of anthocyanin in the cotyledons indicating that there is a lag-phase of few hrs. before appreciable amounts of precursor are synthesized and translocated. Malaviya and Laloraya (1966) came to a similar conclusion working on Celosia seedlings.

More recently, Pecket and Small (1980) studied the site of anthocyanin synthesis in red cabbage seedlings and reached the conclusion that the organelles responsible for anthocyanin formation (anthocyanoplasts) are found in the vacuoles of the plant cells and are the sites of anthocyanin biosynthesis.

Beet seedlings are chosen as experimental material in the present study because of our interest in increasing our knowledge about anthocyanin biosynthesis and because of its importance as a crop plant which can accumulate a high concentration of sucrose in the roots. The work also reports an investigation dealing with the physiological effects of n-propanol on anthocyanin synthesis.

MATERIAL AND METHODS

Seed balls of Beta vulgaris cultivar "Bettrave" imported from France were found to be suitable for the present study. The small and big seed balls were sieved out and those retained by 3mm mesh were used as experimental material. Owing to the fact that the coats of these seed balls accumulate germination inhibiting substances, it was necessary to get rid of such inhibitors before germinating the seeds. This was easily achieved by washing and leaching with water, since these inhibitors are known to be water soluble as cited by El-Shishiny and Thoday (1953).

Seven lots of beet seed balls, each lot composed of 50 seeds, were washed and leached with running distilled water for 8hrs which appears to be quite sufficient to eliminate the germination inhibitory substances in the coats of beet seed balls. After washing, seed balls were removed and rinsed in 70% ethyl alcohol for surface sterilization (Hatata and Shehata, 1979) washed several times with sterile distilled water, then distributed in large sterilized Petri-dishes containing two filter papers moistened with 20ml sterile distilled water. In experiments using n-propanol two days old seedlings were transferred to Petri-dishes containing 20ml of different concentrations of n-propanol. It was found that with the age of seedlings employed no significant damage was done to the material during this operation. The dishes were transferred to a lightened thermostatically-controlled incubator at constant temperature of 25°C and constant light intensity of 6,000 lux.

After 4 days of incubation, the beet seedlings were ready to be used as experimental material to study the effect continuous illumination and different concentration of n-propanol on anthocyanin formation. The same technique in germination, incubation and concentrations of propanol was used under continuous darkness and the Petri-dishes were wrapped with black polythene to prevent any exposure to light during the experiment. Hypocotyls and cotyledons were separated and anthocyanin intensity was determined daily from the 4th to the 12th day of growth. Anthocyanin pigment was extracted using 1% HCl (Muraveva et al. 1987). Anthocyanin assay was carried out by selecting daily samples starting from the 4th day of germination of the seeds till the 12th day of growth under continuous light or continuous darkness. Each sample was composed of comparable 10 seedlings from the dishes.

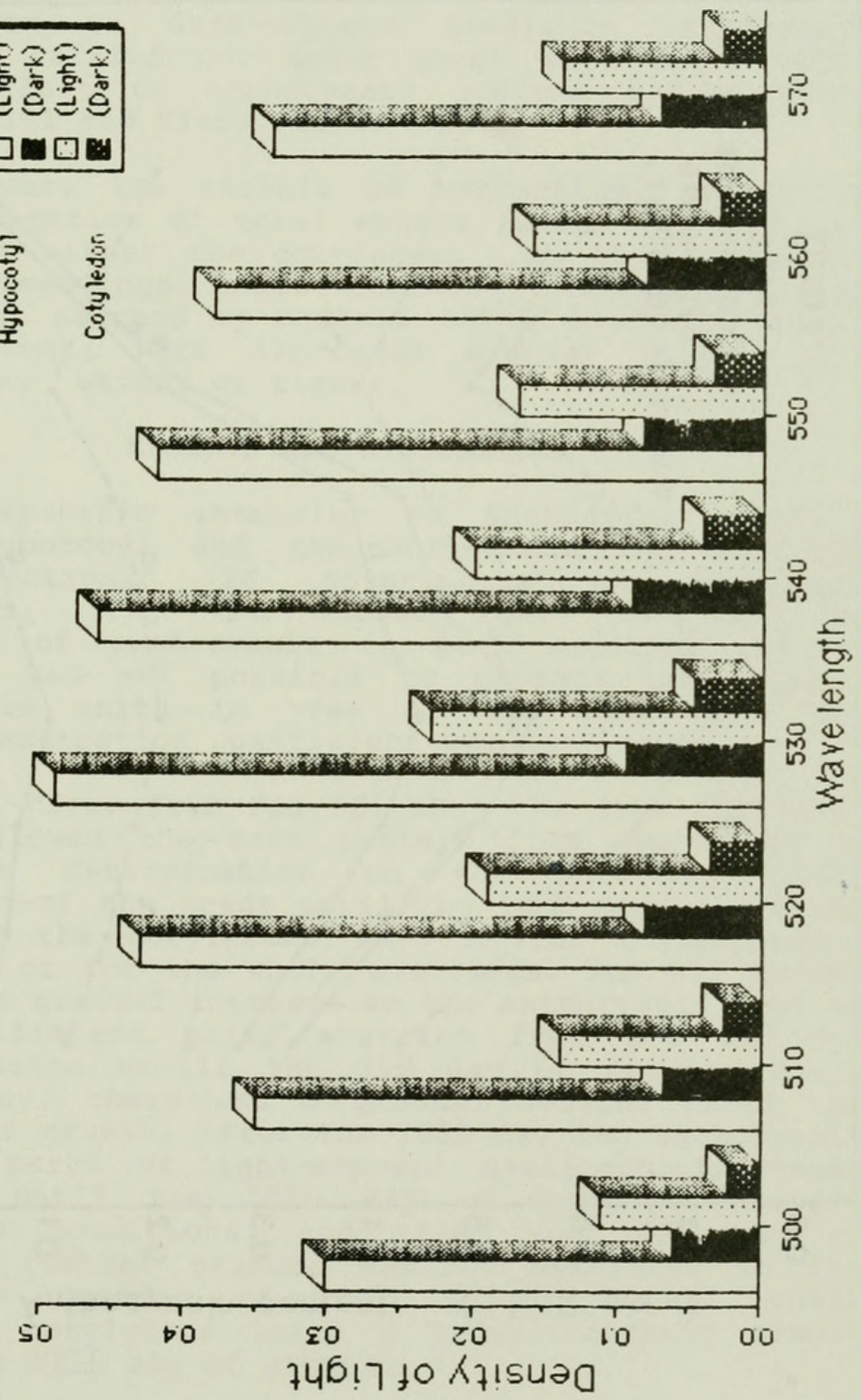

Fig. (1) Anthocyanin density of beet seedlings at different wave lengthes (light & dark)

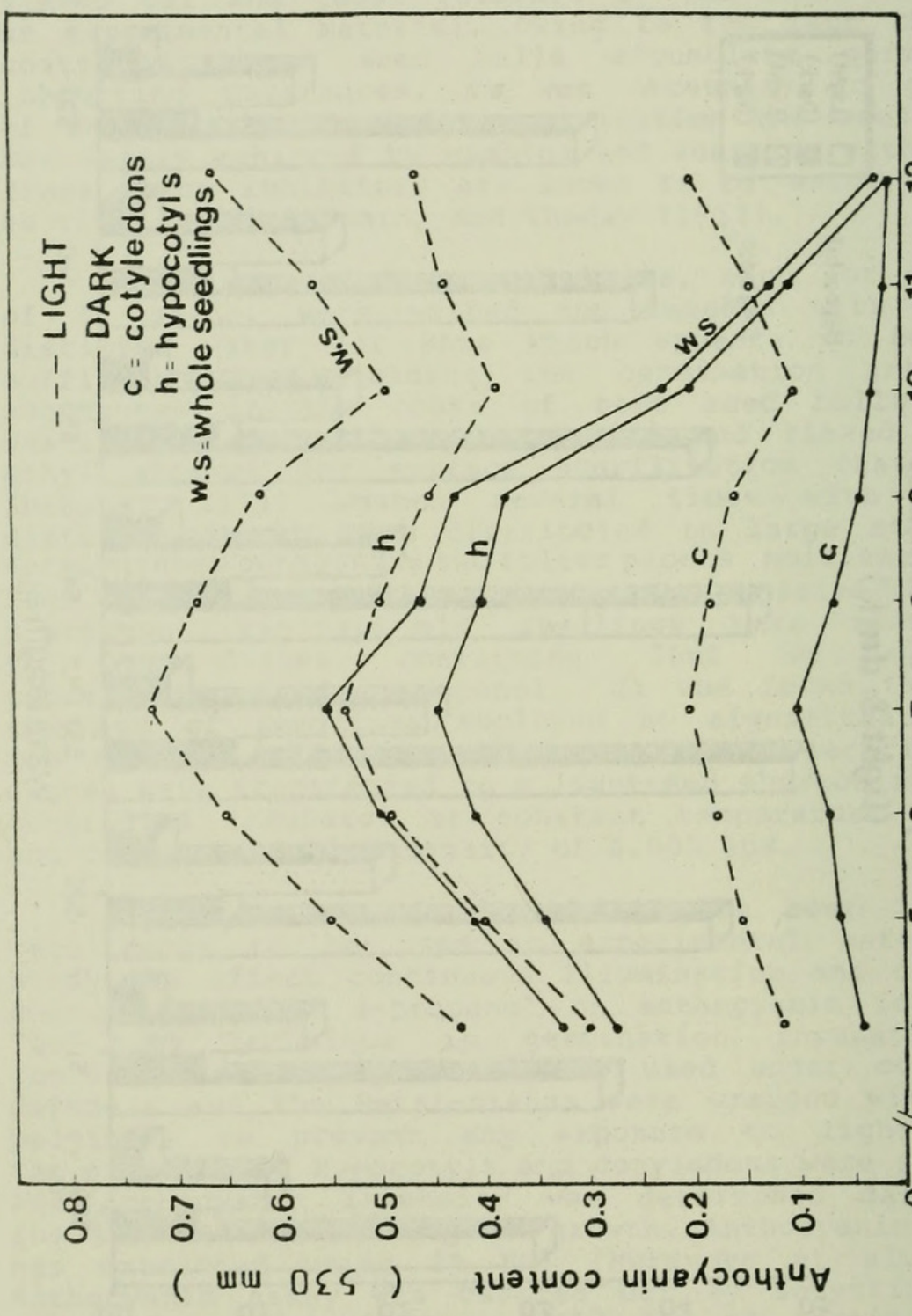

Fig. (2) Specific density of anthocyanin content of beet seedlings

For every sample, the hypocotyls of the 10 seedlings were separated from the cotyledons, then 10ml of 1% aquous HCl was added to each lot in three successive aliquots of 5,3,2 ml for anthocyanin extraction (Pecket and Hathout, 1974).

However in dark-exposed seedlings anthocyanin assay was carried out under green safe light which has no effect on anthocyanin content during assay period (Grill and Vince, 1964).

Moreover, the technic of extraction, separation and determination of total sugars (using the anthrone method) in either the cotyledons or the hypocotyls of beet seedlings under different treatments were the same as adopted by Nosseir (1968) where the total sugar contents were expressed as mgm glucose per 100 gram dry weight of tissue.

RESULTS AND DISCUSSION

The specific intensity of anthocyanin content of the hypocotyl and the cotyledons was measured using spectronic 20 colorimeter at different wavelengths. Fig (1) showed that the peak of absorption of anthocyanin in beet seddling was at 530mm. It was not possible to express the pigment in absolute units in view of the fact that its molecular extinction coefficient was not known.

It is clear from Fig (2) that the dark and light curves followed the same pattern from the start of anthocyanin determination on the 4^{th} day after germination of the seeds until the 10^{th} day of growth, either for the cotyledons, or for the corresponding hypocotyl, or for the whole seedlings. For all curves there was a gradual increase in the anthocyanin content of the different parts starting from the 4^{th} day of germination untill the 7^{th} day of growth. After the 7^{th} day, there was a gradual decline until the 10^{th} day of growth. After the 10^{th} day the anthocyanin level of parts of light-exposed seedlings increased gradually until the 12^{th} day of growth. However, under dark conditions, and after the 10^{th} day of growth, a further gradual decline persisted in the anthocyanin content of both hypocotyl and their respective cotyledons until a value reaching almost zero on the 12^{th} day of growth.

Anthocyanin content of the hypocotyl was always higher than those of the corresponding cotyledons either in light or dark but the content of the light exposed seedlings was higher than those of the dark exposed ones Fig. (2).

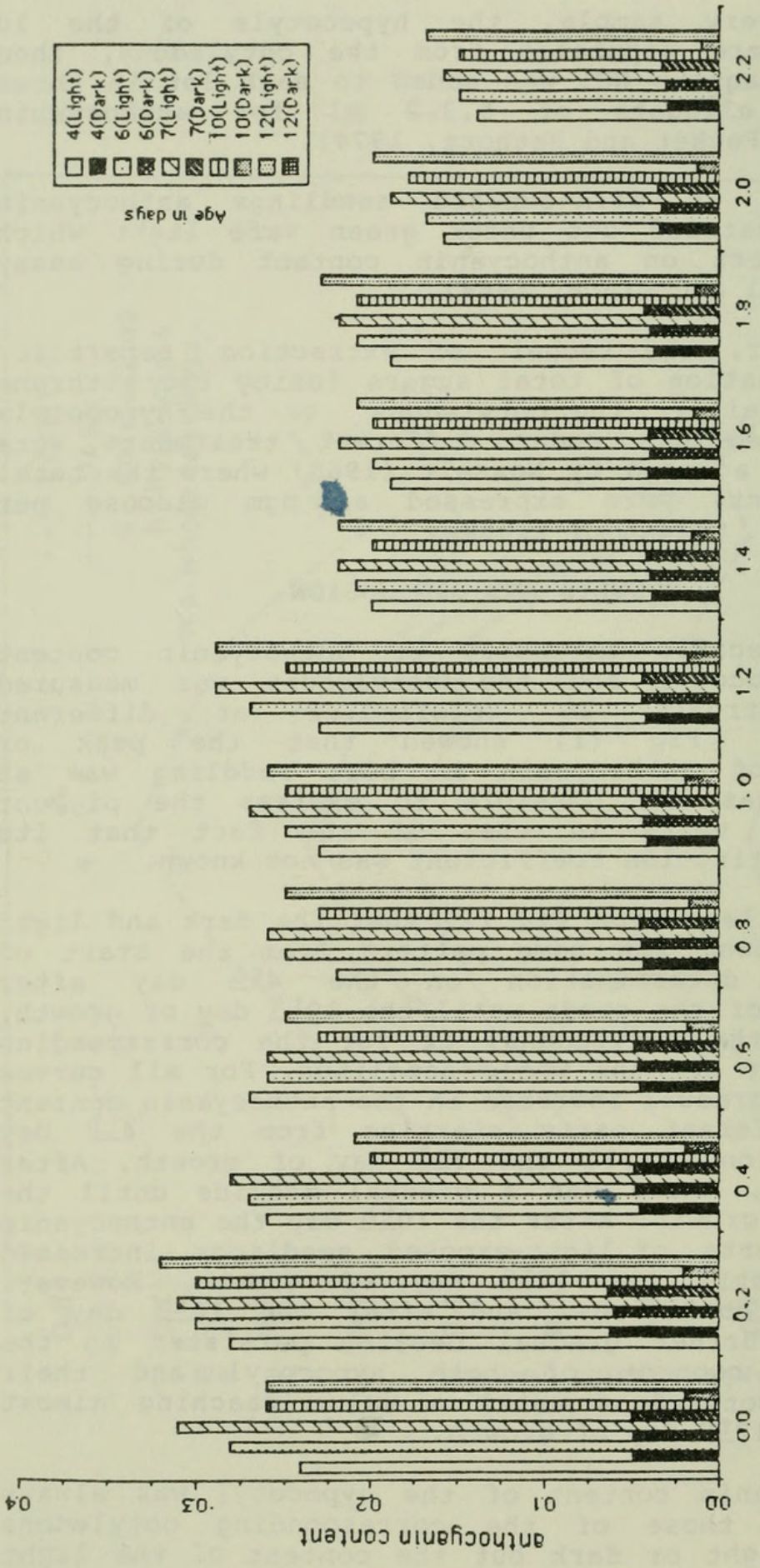

Fig. (3) Effect of different conc. of n-propanol on the anthocianin formation in the cotyledons of beet seedlings (grown in light & dark)

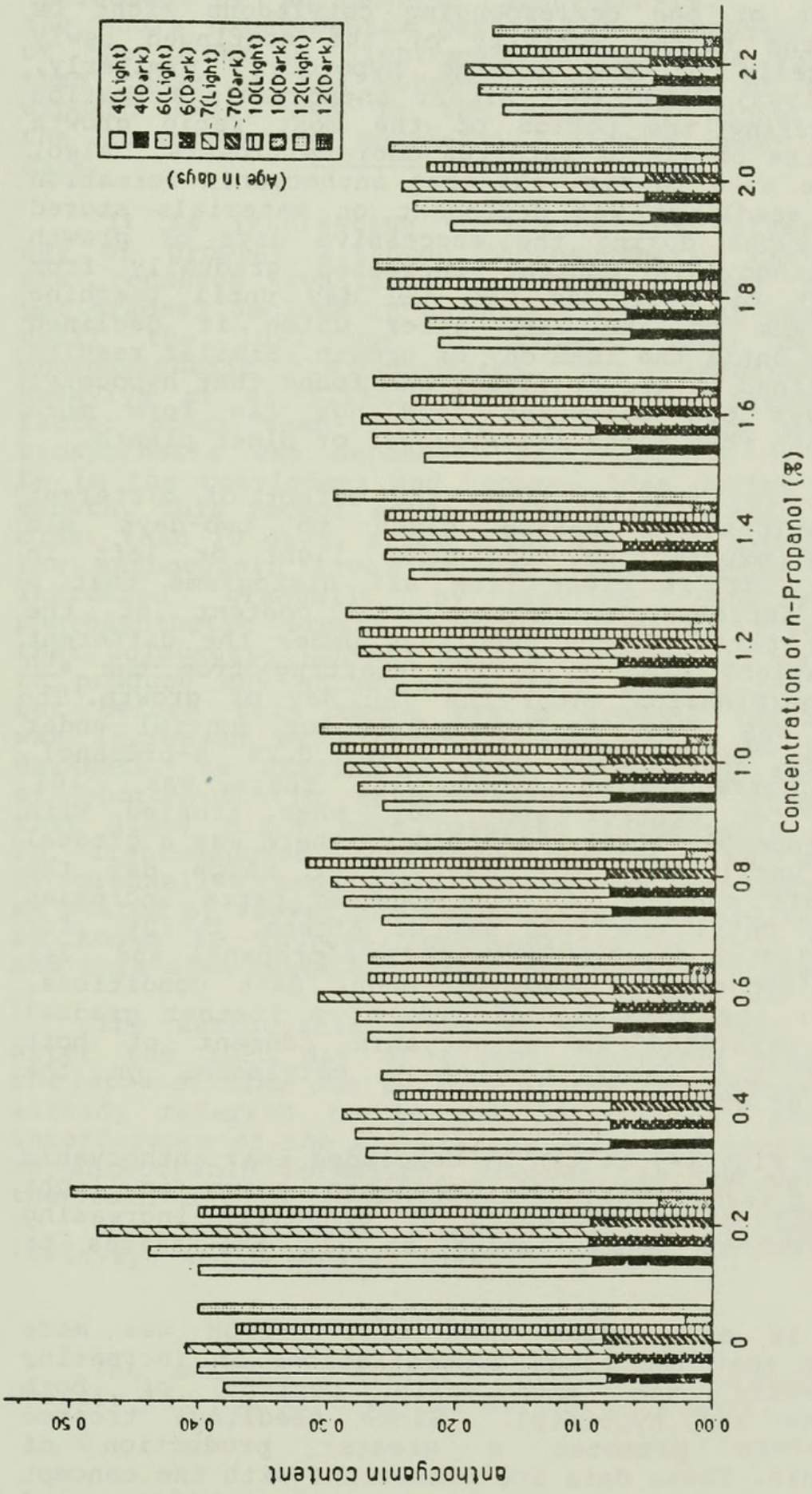

Fig.(4) Effect of different conc. of n-propanol on the anthocyanin formation in the hypocotyl of beet seedlings (grown in light & dark)

The high content of anthocyanin in hypocotyl over that of the corresponding cotyledons might be due to the higher activity of the continued newly formed cells of the growing hypocotyl. Similarly, Pecket (1965) found that higher anthocyanin formation occurs during the period of the most rapid growth rate of the petals of Lathyrus odorata flowers. Also, it can be seen in Fig. (2) that anthocyanin formation in beet seedlings was dependent on materials stored in cotyledons during the successive days of growth where anthocyanin content increased gradually from the start of assay on the 4th day until reaching its maximum on the 7th, after which it declined gradually until the 10th day of growth. Similar results were obtained by Troyer (1964) who found that hypocotyl of 7 days old buckwheat seedlings can form more anthocyanin than those from younger or older plants.

Fig. (3) and (4) shows the effect of different concentrations of n-PrOH given to two-days old seedlings exposed to continuous light or left in darkness. It is clear from all histograms that a gradual increase in anthocyanin content of the different parts of the seedlings under the different concentrations used of n-PrOH starting from the 4th day of germination until the 7th day of growth. The increase was 24% in cotyledons for control under continuous light, and 15% for 0.2% n-propanol. In the corresponding hypocotyls, there was 10% increase for control and 30% when treated with 0.2% n-propanol. After the 7th day, there was a gradual decline until the 10th day, after which day the anthocyanin content of light-exposed parts increased gradually until the 12th day of growth, by 10% for the cotyledons treated with 0.2% n-propanol and 24% for the hypocotyls. However, under dark conditions, and after the 10th day of growth, a further gradual decline persisted in anthocyanin content of both hypocotyl and their respective cotyledons on the 12th day of growth.

From Fig. (4) it can be concluded that anthocyanin content of hypocotyl of seedlings grown in light was significantly decreased by gradually increasing the n-propanol concentration at all the stages of growth.

It is also clear that 0.2% n-PrOH was more effective than the other concentrations in increasing significantly the anthocyanin content of both cotyledons and hypocotyl. Since seedlings treated with n-PrOH promoted a greater production of anthocyanin. These data are consistent with the concept that n-PrOH increase membrane permeability and facilitates the entry of substrate to the site of antocyanin biosynthesis (Pecket and Small, 1980). Table (1) and (2) showed that the total sugars was significantly

increased by anthocyanin biosynthesis. Throughout the whole experimental period the anthocyanin content of the parts of light-exposed seedlings were higher than those of the corresponding parts of the dark-exposed ones, and in the hypocotyl higher than in the corresponding cotyledons, either under light or dark.

It is interesting to notice that after the 7th day of growth, although photosynthetic carbohydrates are rendered available in light-exposed seedlings, yet gradual decline in anthocyanin content was apparent until the 10th day of growth. Such decline in photosynthetic carbohydrates was interpretted by Malaviya et al (1966) as due to the presence of a factor other than carbohydrates on which anthocyanin biosynthesis was dependent and this factor seems to be in the cotyledons and becomes lost during continued growth. This factor seems to be lost in beet seedlings older than 10 days, since after the 10th day of growth, the anthocyanin level of the light-exposed seedlings increased gradually by further development of photosynthetic organs. Malaviya et al (1966) found that Colocassia plumosa seedlings grown in dark failed to produce any anthocyanin after 5 days in darkness, but before this there was a gradual decline which was increased by elongating the period of growth in darkness. He attributed such decline to the absence of photosynthetic carbohydrates. This study showed that light might have promoted anthocyanin formation in light-exposed seedlings through its activation of organelles responsible for anthocyanin biosynthesis as stated by Pecket and Small (1980). In addition, light is known to increase permeability of cell membranes and this also might activate anthocyanin biosynthesis.

The anthocyanin level of light-exposed seedlings after the 10th day of growth increased gradually by the lapse of time due to loss of the cotyledonary factor already referred to in this discussion, and to the interference of the stimulating effect of photosynthesis on anthocyanin formation. However, in the dark, after the 10th day of growth, the gradual decline was due to the continuous consumption of photosynthates (Malaviya and Laloraya, 1966).

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Table (1): Effect of different conc. of n-propanol on the total sugars of the cotyledons of beet seedlings (light & dark) (mg glucose/100 g.d.wt.).

Age in days / Treatment		conc. of n-propanol (%)												L S D	
		0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	5%	1%
4	dark	80.9	83.7*	72.9**	78.3*	76.4**	75.9**	75.6**	76.1**	75.3**	75.0**	72.1**	71.9**	2.26	3.29
	light	287.4	306.1**	297.2**	281.6*	280.9*	266.4**	278.3**	272.6**	252.6**	246.4**	242.3**	230.1	5.13	7.46
6	dark	81.2	84.4**	80.3	77.9**	75.9**	76.1**	75.9**	76.2**	76.6**	75.4**	73.2**	72.6**	2.05	2.98
	light	293.9	321.3**	304.3**	287.8*	288.1*	281.3**	281.2**	290.6	261.3**	258.1**	251.2**	239.8**	5.42	7.88
7	dark	83.6	98.7**	83.8	80.8*	80.6*	76.8**	76.3**	77.0**	76.8**	76.1**	74.6**	73.1**	1.93	2.81
	light	301.2	432.9**	322.4**	303.6	300.6	288.7**	294.9*	300.8	289.3**	280.2**	258.3**	242.1**	5.73	8.33
10	dark	79.8	92.6**	80.6	79.3	76.9**	76.6**	76.4**	76.8**	75.1**	74.8**	71.8**	71.0**	1.91	2.78
	light	280.6	382.7**	303.8**	291.8**	281.6	281.1	278.2	272.9*	260.6**	259.9**	242.6**	236.2**	5.69	8.28
12	dark	62.7	63.6	63.9	63.6	63.1	64.9*	64.6*	64.9*	64.7*	65.9*	65.1*	64.6*	1.89	2.75
	light	293.2	430.6**	306.3**	302.9**	299.3	292.6	281.1**	291.3	288.6	288.3	250.3**	239.1**	5.67	8.25

* Results significantly different from control at the 5% level.
** Results significantly different from control at the 1% level.

Table (2): Effect of different conc. of n-propanol on the total sugars of the hypocotyl of beet seedlings (light & dark) (mg glucose/100 g.d. wt.).

Age in days	Treatment	conc. of n-propanol (%)												L S D	
		0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	5%	1%
4	dark	91.3	104.1*	103.8*	93.2	92.7	92.7	89.4	89.3	89.4	88.7*	78.4**	76.7**	2.47	3.59
	light	456.9	521.8**	521.6**	475.2**	454.8	450.6	446.3**	451.6	451.9	450.3	376.2**	353.1**	7.18	10.44
6	dark	92.4	109.7**	103.8**	93.1	92.7	92.6	90.6	90.9	90.7	89.6*	80.8**	77.3**	2.51	3.65
	light	469.8	546.7**	527.4**	527.3**	470.9	460.8*	451.8**	458.4**	474.3	470.9	383.1**	354.6**	7.07	10.28
7	dark	94.5	115.8**	106.2**	93.9	92.3	92.9	91.2*	91.9*	92.8	92.1	81.4**	78.6**	2.46	3.58
	light	475.7	698.4**	531.6**	532.0**	477.2	478.1	477.9	480.3	482.1	480.9	444.3**	408.1**	7.46	10.85
10	dark	56.2	61.8**	57.2	56.7	81.4**	81.7**	80.8**	80.6**	80.3**	80.9**	71.6**	69.4**	2.67	3.88
	light	471.6	610.7**	527.5**	527.5**	475.1	469.7	467.9	475.6	476.2	474.6	409.8**	408.3**	7.43	10.81
12	dark	46.6	48.7*	45.7	45.2	46.3	46.0	46.1	45.7	45.1	44.8	43.2**	42.6**	1.98	2.88
	light	472.8	699.3**	530.9**	511.4**	461.7*	450.1**	437.2**	446.8**	442.5**	442.2**	375.6	474.6	8.10	11.78

* Results significantly different from control at the 5% level.
** Results significantly different from control at the 1% level.

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