ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXVIII — Nr 3
1959

Antyhocyanin synthesis in isolated embryos of red cabbage and radish

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The *in vitro* culture of isolated plant tissues, organs and embryos has proved to be a valuable method in studying many problems of plant physiology, especially of plant metabolism. Among others the method has proved suitable in studying the physiology of anthocyanin pigment formation. In some previous papers we used the isolated grape tissue (Szweykowska 1952 and 1955). The present paper gives our results of investigating the influence of some more important constituents of the medium on anthocyanin synthesis in isolated embryos. The method of embryo culture has the advantage of ommitting the transplantation procedure and having a fresh experimental material at any time.

MATERIAL AND METHODS

Embryos of red cabbage var. Kisendrupp and of radish var. Saxa were used in the experiments. The seeds were sterilized by 10 min. immersion in $2^{\circ}/_{00}$ $HgCl_2$ and after repeated washing soaked in distilled water for 18 hrs. Then the seed coat and cotyledons were removed and the embryos (three per tube) placed on the surface of an agar medium. The basal medium was a Knop solution (2 × diluted) with sugar and agar added. This medium was modified variously in the experiments. The cultures of red cabbage were grown during 7 days in a thermostat at 25°C in the dark. In the radish seedlings light is necessary for the formation of pigment and therefore, after 3 day incubation in the dark, they were transferred into the light of white fluorescent tubes at an intensity of 2.100 lux and temp. 23°C. In each experimental series 72 embryos growing in 24 tubes were used. About 50 of them were used in analyzes. The roots were cut off, the hypocotyls weighed and then ground in a $1^{0}/_{0}$ solution of HCl in methanol. The red anthocyanin solution was centrifuged and, in the case of light growing radish embryos which had become slightly green, shaked with petroleum ether to remove chloroplast pigments. The clear extract was measured in Pulfrich apparatus with S 53 filter (transmittance max. 5.300 A). The standard curve for red cabbage pigment was obtained by using rubrobrassicin prepared by the method of Chmielewska (1938). Our efforts of preparing the anthocyanin pigment from radish root (an acylated derivative of

pelargonidin-3-diglucosido-5-monoglucosid, Harborne and Sherrat 1957) were unsuccessful. The amount of this pigment was expressed in pigment units. A solution containing 100 units/ml gave a light transmission = 8%.

RESULTS

The effect of sucrose. 4% sucrose was optimal for the growth of both cabbage and radish embryos. Above this concentration the fresh weight of seedlings gradually diminshes (fig. 1). On the contrary, anthocyanin synthesis increases begin-

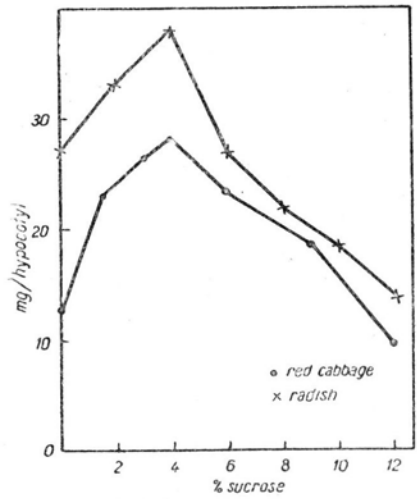

Fig. 1. Effect of sucrose on the growth of hypocotyls

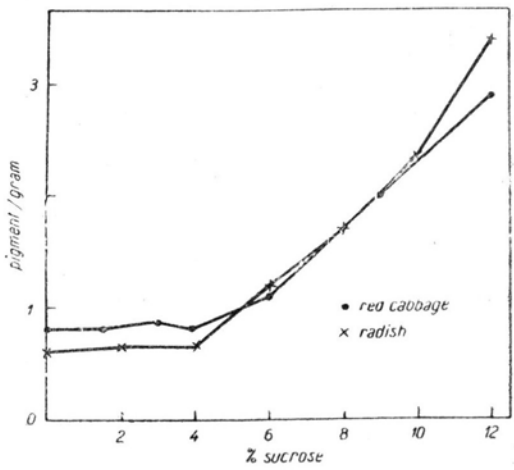

Fig. 2. Effect of sucrose on the pigment concentration in seedlings

ning with a 4% concentration of sucrose in the medium (fig. 2). The fresh weight decrease of a plant tissue cultivated on higher sugar concentrations is caused to a great extent by its diminished water content (Szweykowska 1952). However, the cell sap concentration is not the only reason of higher anthocyanin content in the tissue. This is shown by an increasing pigment production when calculated as pigment amount per seedling (fig. 3).

The effect of various sugars. The effects of glucose, fructose, mannose, sorbose, galactose, xylose, arabinose, sucrose, maltose, lactose and raffinose on embryos of red cabbage were investigated. The concentration in the medium varied from 0,044 to 0,350 M. In the case of sucrose it corresponds to 1,5—12% (w/vol.). Arabinose, xylose, galactose and sorbose had a harmful effect on embryo development. When feeding with these sugars the embryos died after 3 days of cultivation. The influence of other sugars tested on growth and anthocyanin synthesis in hypocotyls of red cabbage is shown in table 1.

Sucrose had the best effect on the growth of embryos. The embryos grew well also on glucose, maltose and lactose. Fructose at higher concentrations had a harmful effect on the development of embryos and caused their death. The growth of embryos on raffinose was rather feeble. The feeding with mannose caused a decrease of the fresh weight beginning with lowest concentrations used, but the sugar is not toxic and even in higher concentrations the embryos remained alive.

With reference to anthocyanin synthesis fructose had a distinctly decreasing effect on this process. It may be stated, that together with the above pointed unfavourable effect of fructose on growth it is toxic for cabbage seedlings. Of the remain-

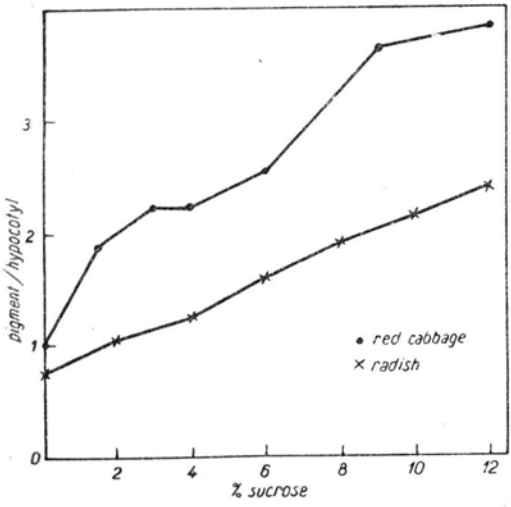

Fig. 3. Effect of sucrose on pigment formation calculated „per seedling"

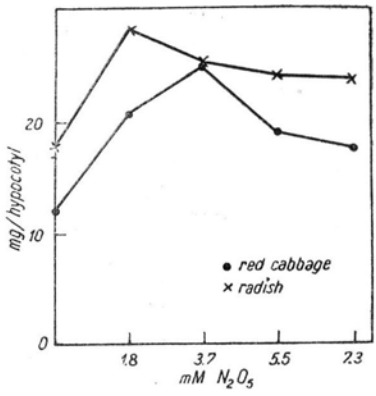

Fig. 4. Effect of nitrogen feeding on the growth of hypocotyls, in a medium with 4% sucrose

ing sugars mannose and raffinose were most effective in promoting anthocyanin formation. Maltose, glucose and lactose promoted pigment production only when used in higher concentrations. The effect of sucrose was similar but the increase of anthocyanin production was here relatively low.

All facts presented above indicate some contrast between growth (expressed in fresh weight) and anthocyanin formation. The sugars which had the best effect on growth promoted pigment production to a relatively lower extent than those whose effect on growth was more feeble (sucrose on the one side and mannose and raffinose on the other; the effect of glucose, mannose and lactose on growth and pigment formation was intermediate between that of the sugars cited above).

Effect of nitrogen and phosphor. Nitrogen was added to the medium in form of nitrates. In media lacking nitrogen the NO_3' anion was replaced by a Cl' anion. Extra nitrogen was given in form of $NaNO_3$.

Table 1

The influence of various sugars on fresh weight and anthocyanin content in red cabbage hypocotyls

Concentration M	Concentration %	Sugar	Fresh weight mg/seedling	Anthocyanin mg/g fresh wt.
0,000	0,0	—	13	0,8
0,044	2,2	raffinose	16	1,1
0,088	1,6	glucose	24	1,0
		fructose	23	0,7
		mannose	9	1,4
	3,0	sucrose	29	1,1
		maltose	19	1,0
		lactose	23	1,0
	4,4	raffinose	17	1,0
0,175	3,2	glucose	22	1,0
		fructose	19	0,8
		mannose	7	2,0
	6,0	sucrose	26	1,0
		maltose	21	1,0
		lactose	16	1,0
	8,8	raffinose	13	1,5
0,350	6,3	glucose	8	3,9
		fructose	died	—
		mannose	4	4,1
	12,0	sucrose	15	2,8
		maltose	7	4,5
		lactose	4	3,7

Table 2

Effect of nitrogen feeding on growth and anthocyanin concentration in seedlings of red cabbage, in a sugar-less medium

N_2O_5 mM/l	Fresh wt. mg/seedling	Anthocyanin mg/g fresh wt.
0,0	11,0	1,0
1,8	12,4	0,8
3,7	12,3	1,0
5,5	13,2	0,9
7,3	13,2	0,9

In the absence of sugar nitrates had no effect on pigment formation nor on the fresh weight of seedlings (table no. 2). In other experiments 4 and 8% sucrose media were used. The effect of nitrogen feeding on the fresh weight of hypocotyls is shown in fig. 4. In the absence of nitrogen an increased anthocyanin formation occured,

but beginning with the lowest concentration used (which was that of optimal for growth) its increased amount had a very slight effect on pigment production (fig. 5).

The influence of phosphorus feeding (KH_2PO_4 and NaH_2PO_4) was investigated only for red cabbage embryos. The effect of phosphates on growth as well as on

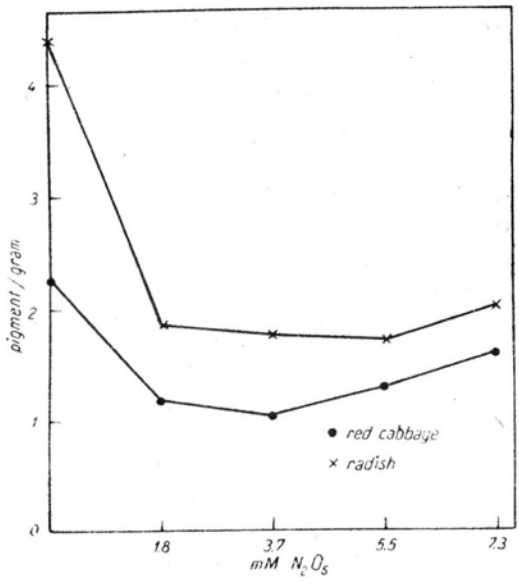

Fig. 5. Effect of nitrogen feeding on pigment concentration in seedlings, in a medium with 4% sucrose

Table 3

Effect of phosphorus feeding on growth and anthocyanin production in seedlings of red cabbage, in a medium with 4% sucrose

P_2O_5 mM/l	Fresh wt. mg/seedling	Anthocyanin mg/g fresh wt.
0,0	20	1,2
2,3	22	0,9
4,6	20	0,9
5,7	20	1,0
6,9	23	0,8
8,1	23	0,8
9,2	22	0,9

anthocyanin synthesis is but insignificant. The lack of phosphorus causes a feeble increase of the amount of pigment (table 3).

DISCUSSION

An anthocyanin synthesis in hypocotyls of seedlings which had been deprived of cotyledons shows that the hypocotyls themselves possess the ability of synthesizing the pigment and thus no precursor from cotyledons is needed.

The distinct effect of sugar on this process has been established in some former as well as in many recent investigations (Overton 1899, Thimann and Edmondson 1949, Eddy and Mapson 1951, Szweykowska 1952). Our experiments with plant embryos are only a further illustration of this fact. But some facts characteristic for conditions of *in vitro* cultures (Szweykowska 1952) must be emphasized. Sugar promotes anthocyanin synthesis only when used in concentrations above those optimal for growth (fig. 1—2). With reference to the influence of various sugars each of them (excepting fructose) stimulates pigment synthesis the more the greater the decrease of growth in the hypocotyl.

The results obtained differ from those of Eddy and Mapson (1951) in that sorbose, galactose, xylose and arabinose do not increase the pigment content of the seedlings and are toxic for them. Obviously these sugars are not able to enter into the normal pathway of embryo metabolism and cause metabolic disturbances leading soon to the death of the embryos.

The negative effect of nitrogen on anthocyanin formation in plants has been observed by several authors (Czartkowski 1914, Reinhold and Kochs 1935, Gassner and Straib 1937, Eddy and Mapson 1951). Paech (1956) stated a similar fact in respect to carotenoids, i.e. an increased nitrogen supply caused a decrease in the amount of carotenoids in etiolated wheat seedlings. Paech's, interpretation is that a competition for sugars exists between the protein and carotenoid metabolisms. This interpretation is supported by the fact that nitrogen feeding has no effect on the pigment amount in seedlings that are grown on sugarless media. This influence is therefore undoubtedly connected in some way with carbohydrate metabolism.

SUMMARY

1. The influence of sugar and nitrogen feeding on growth and anthocyanin synthesis in isolated embryos of red cabbage and radish was investigated. Morevoer, using red cabbage embryos also the influence of 11 various sugars and of the feeding with phosphorus was investigated.

2. Arabinose, xylose, galactose and sorbose have a harmful effect on embryo development and cause their death after a few days of incubation. Fructose acts similarly in higher concentrations. In higher concentrations the remaining sugars (glucose, mannose, sucrose, maltose, lactose and raffinose) promote the more anthocyanin formation the more they inhibit growth expressed as fresh weight per seedling, in the following order: mannose, raffinose, maltose, glucose, lactose, sucrose.

3. The promoting effect of sugar feeding on pigment production occurs only in concentrations of sugar higher than that optimal for growth. Above this con-

centration the pigment amount in seedlings is proportional to the extra-sugar in the medium.

4. The absence of phosphates in the medium results in a slight increase of the pigment amount.

5. The absence of nitrogen results in an increase of pigment in the seedlings, provided the sugar concentration is an optimal or supraoptimal one. Nitrogen feeding in the absence of sugar has no effect on pigment synthesis. This indicates a relationship between this process and sugar metabolism.

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