BIOLOGIA PLANTARUM (PRAHA)

12 (3) : 231—235, 1970

Effect of Phosphorus Deficiency on Anthocyanin Content in Tomato Plants

MARIE ULRYCHOVÁ and VĚRA SOSNOVÁ

Department of Plant Pathology, Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha

Received September 28, 1969

Abstract. The effect of phosphorus deficiency on anthocyanin content in tomato plants was studied from the point of view of pathological anthocyanin formation. Phosphorus deficiency resulted in an evident increase of anthocyanin content in all experiments, independently of the tomato variety and of the cultivation conditions. The anthocyanin amount was five times higher on the average and the differences were statistically significant. The average growth inhibition of tomato plants under phosphorus deficiency was 18%. Accumulation of anthocyanins is limited by the temperature; anthocyanins are not synthesized at temperatures above 30°. The results of the experimental variant with enhanced phosphorus concentration in nutrient solution were not uniform. In some experiments a decrease of anthocyanins accompanied by slight growth stimulation was observed, in others an increase associated with growth inhibition. It seems that cultivation conditions, especially the light intensity, play an important role in these experimental variants.

Biosynthesis of anthocyanins in connection with virus infection was first studied in detail by MARTIN (1958). This author compared anthocyanin formation in healthy and Y-virus-infected young potato plants of the variety Bintje and found an indirect correlation between virus and anthocyanin synthesis. If Martin's results are compared with the conclusions of THIMANN and RADNER (1955, 1962), who suppose that anthocyanin formation is controled by an unstable nucleic acid which must be continually resynthesized, a hypothesis of competition between the above mentioned unstable nucleic acid and the virus nucleic acid is offered. The work of ULRYCHOVÁ and BRČÁK (1967) who reported an inhibitory effect of anthocyanins on TMV reproduction is also in agreement with this hypothesis.

The relation between virus infection and anomalous anthocyanin formation in plants attacked by viruses is not such a simple function as the facts mentioned would indicate. It is known that anthocyanin formation in plants infected with potato leaf roll virus varies greatly depending on the variety, on the vegetation period and from one year to another. The reasons

* *Address*: Na Karlovce 1, Praha 6, Czechoslovakia.

for these differences in anthocyanin formation are probably complicated relations with a number of other factors.

THIMANN and EDMONDSON (1949) reported in the first of a series of very exact papers concerning anthocyanin biosynthesis in *Spirodela oligorrhiza*, that light is an indispensable factor for anthocyanin formation and that sugars, especially saccharose, have a strong stimulatory effect. Accumulation of sugars in potato leaf roll virus infected plants is well known and a detailed dynamic study in this respect was carried out by PERDRIZET and MACQUAIRE (1963) and VERHOEKS (1965).

A number of recent papers are devoted to the study of anthocyanin synthesis induced by different substrates (HESS 1967, 1968, SCHRAUDOLF 1967). If the evident physiological changes owing to the presence of virus or another infectious agent in the host plants are taken into consideration, the possibility of a substrate-induced anthocyanin synthesis in virus infected plants becames very probable.

An important contribution to the study of factors conditioning anthocyanin synthesis is the paper of PAYNOT and MARTIN (1968). These authors observed the dependence of anthocyanin synthesis in leaves of *Begonia gracilis* on the temperature (17°, 20°, 23°, 30°) under constant illumination of 8000 lux and 16 hours' photoperiod. The most intense anthocyanin synthesis took place at the temperature 17°; when the temperature was raised the intensity of anthocyanin formation decreased and was practically stopped at 30°. This conclusion can be connected with the commonly known observation of a stronger anthocyanin formation in cold weather.

HOVELAND (1954) has found a reduced phosphorus content in leaves of potato plants, variety Chippewa, infected with leaf roll virus. This author proved in addition that phosphorus deficiency provokes in potato plants of the corresponding variety symptoms similar to leaf roll infection *i.e.* severe rolling of the leaves and heavy anthocyanin formation. ULRYCHOVÁ and LIMBERK (1967) have found that an intense anthocyanin formation and a decrease of total phosphorus up to 28% of control took place in the leaves of the hypersensitive resistent variety of potato plant Apta after leaf roll virus infection. ULRYCHOVÁ and LIMBERK (1968) have further stated that in potato varieties particularly susceptible to leaf roll, there was a decrease of total phosphorus after infection. The given facts indicate that pathological anthocyanin formation can be intimately connected with phosphorus deficiency. The aim of our present paper was to verify this relation.

Material and Methods

Plant Cultivation

We used tomato plants of the variety Imun in most experiments; in two experiments, tomato plants designated 63L 1245* from California were used and in one experiment, we worked with the variety Průhonické. The experiments were carried out partly in a greenhouse partly in a cultivation

* Seeds were obtained from the Department of Vegetable Crops, University of California.

box with artificial illumination by two 500 W Philips mercury lamps and a twelve hour photoperiod and at an average temperature of 20°.

Germination of seeds and cultivation of seedlings were carried out in pots filled with small glass balls. The pots with control plants were watered with Knop's nutrient solution and the experimental variants with Knop's nutrient solution either without phosphorus or with double phosphorus dose.

Anthocyanin Estimation

Anthocyanin content in tomato plants was determined five weeks after the establishment of an experiment. Extraction of the pigments was carried out with 0·2N perchloric acid; 10 g of material were thoroughly homogenized for several minutes with 100 ml of acid. The homogenate thus obtained was centrifuged at 10 000× *g* for 20 minutes and the supernatant was filtred through a dense paper filter (Schleicher et Schuell No. 589³). THIMANN and EDMONDSON (1949) extracted the material with 0·1N HCl and WAGNER and MOOHR (1966) with the mixture propanol, HCl and water. They carried out heat extraction during several hours. Because our method is very simple, rapid and quantitative according to our preliminary experiments, we preferred it to the published methods described. The color intensity was measured on a Pulfrich colorimeter with Elpho supplement using the filters S_{53} and S_{65}. Correction of light dispersion was made according to WAGNER and MOHR (1966). The effect of phosphorus deficiency on the growth of plants was determined by weighing 20 plants.

Results and Discussion

The average growth inhibition of tomato plants subjected to phosphorus deficiency was 18·1% and this delay in growth was statistically significant ($P > 0{\cdot}05$). Increased phosphorus concentration in nutrient solution did not exhibit a distinctly uniform effect; in some experimental series there was a stimulation, in others an inhibition of growth. In average, a statistically insignificant growth inhibition of 8% was found.

Anthocyanin production in the different experimental series and variants is given in the Table 1.

As can be seen from the Table 1, phosphorus deficiency resulted in evident increase of anthocyanin level in all experiments, independently of the tomato variety and of the cultivation conditions. Anthocyanin content was five times higher on the average and the differences are statistically significant, $P > 0{\cdot}000001$. The average extinction value of the control is 0·15 and that of experimental variant without phosphorus 0·77. Mean difference is 0·62 and standard deviation is $\pm 0{\cdot}32$.

Results of the experimental variant with an enhanced phosphorus concentration in nutrient solution were not uniform, similarly as the growth reaction of these plants. It seems that the intensity of light plays a primary importance in these experiments. In the series where growth inhibition was observed an increased anthocyanin production was found and growth stimulation was accompanied by a decreased anthocyanin level. A final

TABLE 1

EFFECT OF PHOSPHORUS DEFICIENCY and of enhanced phosphorus concentration on anthocyanin formation in tomato plants

Date of experiment	Variety of tomato plant	Cultivation	Anthocyanin content*				
			Control	—P	% of control	+2P	% of control
17. 4. 1968	Imun	Greenhouse	0·12	1·07	892		
20. 5. 1968	Imun	Greenhouse	0·55	1·14	207	0·50	91
25. 6. 1968	Imun	Cultivation box	0·07	0·26	371	0·03	43
18. 10. 1968	Imun	Greenhouse	0·03	0·40	1333	0·08	266
15. 11. 1968	Imun	Cultivation box	0·05	0·45	900	0·04	80
3. 1. 1969	Imun	Cultivation box	0·17	0·99	582	0·39	236
18. 2. 1969	63L1245	Cultivation box	0·14	0·99	707	0·14	100
18. 2. 1969	Imun	Cultivation box	0·04	0·19	475	0·03	75
28. 3. 1969	63L1245	Cultivation box	0·06	0·57	950	0·09	150
28. 3. 1969	Imun	Cultivation box	0·11	1·14	1036	0·17	154
24. 4. 1969	Průhonické	Cultivation box	0·37	1·29	348	0·34	92

* Anhocyanin content is given in extinction values of the extracts obtained by centrifugation and filtration of homogenates (1 g of material and 10 ml of 0·2N $HClO_4$). Measurement was made in 1 cm cell.

conclusion about the effect of enhanced phosphorus concentration in nutrien solution on anthocyanin formation could be made only if experiments were carried out under standard temperature and light conditions.

Evidence for the effect of phosphorus deficiency on anthocyanin accumulation is presented. For the present, we can not say in which metabolic relation this two phenomena are, if there is some direct or indirect relation through the mediation of sugars. Accumulation of anthocyanins under phosphorus deficiency is limited by the temperature. Anthocyanins were not synthesized in summer when the temperature rose above 30°. This observation is in agreement with the results of PAYNOT and MARTIN (1968) who found that anthocyanin synthesis in *Begonia gracilis* stopped at the temperature 30°. Thus the temperature is a factor rating higher than phosphorus deficiency.

Our paper is a contribution to the problem of pathological anthocyanin formation in plants.

References

HESS, D.: Substratinduktion bei der Anthocyan-Synthese von *Petunia*. — Naturwissenschaften **54** : 289—290, 1967.

HESS, D.: Substratinduktionen durch Zimtsäeren als physiologischer Mechanismus bei der Einleitung der Anthocyansynthese. — Z. Pflanzenphys. **59** : 293—296, 1968.

HOVELAND, C. S., BERGER, K. C., DARLING, H. M.: The effect of mineral nutrition on the expression of potato leaf roll virus symptoms. — Proc. Soil. Sci. Amer. **18** : 53—55, 1954.

MARTIN, C.: Etude de quelques déviations de métabolisme chez les plantes atteintes de maladies à virus. — Thèse 1958. Université de Paris.

PAYNOT, M., MARTIN, C.: Composés flavoniques, floraison et hypersensibilité aux virus chez les végétaux. — C. R. Acad. Sci. 266 (Série D): 680—682, 1968.

PERDRIZET, E., MACQUAIRE, G.: Etudes des variations du metabolisme des feuilles de Pomme de terre provoquées par le virus de l'enroulement durant les premier stades de l'infection. — C. R. Acad. Sci. **257** : 3208—3211, 1963.

SCHRAUDOLF, H.: Beeinflussung der Anthocyansynthese in Keimlingen von *Sinapis alba* L. durch α-Methyl-DL-tryptophan. — Planta **77** : 319—324, 1967.

THIMANN, K., EDMONDSON, Y. H.: The biogenesis of the anthocyanins. I. General nutritional conditions leading to anthocyanin formation. — Arch. Biochem. **22** : 33—53, 1949.

THIMANN, K., RADNER, B. S.: The biogenesis of anthocyanins. V. Evidence for the mediation of pyrimidines in anthocyanin synthesis. — Arch. Biochem. Biophys. **59** : 511—525, 1955.

THIMANN, K., RADNER, B. S.: The biogenesis of anthocyanins. VII. The requirement for both purines and pyrimidines. — Arch. Biochem. Biophys. **96** : 270—279, 1962.

ULRYCHOVÁ, M., BRČÁK, J.: Hemmwirkung der Anthocyane auf die Vermehrung des Tabakmosaikvirus. — Phytopath. Z. **58** : 87—91, 1967.

ULRYCHOVÁ, M., LIMBERK, J.: Phosphorus disturbances associated with potato leaf roll virus infection in potato variety Apta. — Plant Virology, Proceedings of the 6th Conference of the Czechoslovak Plant Virologists, pp. 151—156, Olomouc 1967.

ULRYCHOVÁ, M., LIMBERK, J.: Phosphorus disturbances associated with leaf roll virus infection. — Biol. Plant. **10** : 311—317, 1968.

VERHOEKS, J. L.: Photosynthesis and carbohydrate metabolism of healthy and leaf roll diseased potato plants. — H. Veenman et Zonen N. V. Wageningen 1965.

WAGNER, E., MOHR, H.: Kinetische Studien zur Interpretation der Wirkung von Sukzedanbestrahlung mit Hellrot und Dunkelrot bei der Photomorphogenese (Anthocyansynthese bei *Sinapis alba* L.). — Planta **70** : 34—41, 1966.

MARIE ULRYCHOVÁ, VĚRA SOSNOVÁ Ústav experimentální botaniky ČSAV, Praha: **Vliv fosforového deficitu na obsah antokyanů v rostlinách rajčete.** — Biol. Plant. **12** : 231—235, 1970.

V rámci studia patologické antokyanizace u rostlin byl sledován vliv fosforového deficitu na tvorbu antokyanů u rajčat. Nedostatek fosforu vedl ve všech případech nezávisle na použité odrůdě rajčete a na kultivačních podmínkách k výraznému zvýšení hladiny antokyanů. V průměru byl obsah antokyanů zvýšen sedmkrát a rozdíly byly statisticky vysoce průkazné. Průměrná růstová inhibice rostlin rajčat při fosforovém deficitu byla 18%. Hromadění antokyanů bylo limitováno teplotou; při teplotě kolem 30° se antokyany netvořily. Pokusná varianta se zvýšeným obsahem fosforu nedala jednoznačný výsledek. V některých pokusech bylo pozorováno snížení obsahu antokyanů, provázené růstovou stimulací, v některých pak zvýšení, doprovázené naopak růstovou inhibicí. Zdá se, že u této pokusné varianty hrají významnou úlohu kultivační podmínky, zejména intensita osvětlení.