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Effect of Phenolic Acids on Anthocyanin Content in Maize Roots

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Abstract. Supply of 0.01 to 5.0 mM salicylic, caffeic and gallic acids, either during imbibition of seeds for 24 to 48 h or during seedling growth increased anthocyanin production in maize (*Zea mays* L. cv. Ganga safed-2) roots. While tyrosine had no effect, phenylalanine either in the presence or absence of the phenolic acids increased anthocyanin content. Glucose in a concentration range of 1 to 20 mM and shikimic acid in 0.01 to 5.0 mM range also increased pigment level, which was higher in the presence of salicylic acid than in its absence. The experiments demonstrate the possibility of some indirect effects of salicylic acid and other phenolic acids on anthocyanin synthesis.

The regulation of plant growth and metabolism by phenolic compounds has been demonstrated in several systems (VENDRIG and BUFFEL 1961, WAIN and TAYLOR 1965). In our earlier experiments, we recorded that salicylic acid increased growth at low concentrations, while inhibited the same at high (1.0 to 5.0 mM) concentrations. On the other hand, caffeic acid had no effect at low concentrations but inhibited seedling growth at concentrations above 1.0 mM (JAIN and SRIVASTAVA 1981a). Salicylic acid at lower concentrations increased nitrogen content, nitrate reductase and glutamate dehydrogenase activities while it inhibited these parameters at higher concentrations (JAIN and SRIVASTAVA 1981b, c). Salicylic acid also enhances flowering in maize (ASTHANA and SRIVASTAVA 1978), and several other systems. As an extension of these observations, the effect of some phenolic acids on anthocyanin synthesis in maize roots was studied.

MATERIAL AND METHODS

Seeds of *Zea mays* L. cv. Ganga safed-2 were purchased from National Seed Corporation, New Delhi. Seeds were surface sterilized with 0.1% $HgCl_2$ for about 10 min and then washed thoroughly with distilled water. They were placed on moist filter papers in ten cm Petri dishes in continuous light of about 65 W m^{-2} radiant flux density (400—700 nm) supplied by incandescent

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TABLE 1

Effect of phenolic acids on anthocyanin content of maize roots

Conc. of phenolic acids [mM]	Anthocyanin content [A g^{-1}(f.m.)] $\pm$ S.E.		
	Salicylic acid	Caffeic acid	Gallic acid
0.00	1.39 $\pm$ 0.06 (100)	1.39 $\pm$ 0.06 (100)	1.39 $\pm$ 0.06 (100)
0.01	1.70 $\pm$ 0.04 (122)	1.95 $\pm$ 0.07 (140)	2.34 $\pm$ 0.26 (168)
0.10	1.95 $\pm$ 0.02 (140)	1.96 $\pm$ 0.19 (141)	2.30 $\pm$ 0.07 (165)
1.00	1.86 $\pm$ 0.24 (134)	2.44 $\pm$ 0.27 (175)	2.30 $\pm$ 0.06 (165)
5.00	1.84 $\pm$ 0.29 (132)	2.67 $\pm$ 0.24 (192)	2.27 $\pm$ 0.09) (163)

Seedlings were raised either in distilled water or in desired concentration of phenolic acids for four days. Values relative to control are given in brackets.

bulbs and fluorescent tubes at 25 $\pm$ 2 °C. Samples were collected after four days of planting the seeds (including imbibition period). Phenolic acids were supplied either by imbibing the seeds for 24 or 48 h in light, in solutions of desired phenolic acid (exp. 2) or by moistening the filter paper with the desired solution (exp. 1, 3—5).

Anthocyanin content in the primary roots was extracted and determined by the method of MANCINELLI and WALSH (1979). The roots (5 in each case) from uniformly grown seedlings were weighed and cut into small pieces of about 2 mm. These segments were then suspended in acidic methanol (1% HCl in absolute methanol) for 48 h at 10 °C. The absorbance of the extract was determined in a colorimeter using a green filter. The data presented are average $\pm$ S.E. of at least six replicates.

RESULTS AND DISCUSSION

Supply of salicylic acid, caffeic acid and gallic acid during seed germination and seedling growth increased anthocyanin content as compared to control, in maize roots. The effect of caffeic acid was concentration dependent *i.e.* by

TABLE 2

Effect of phenolic acids, supplied to seeds during imbibition period, on anthocyanin content

Phenolic acids	Anthocyanin content [A g^{-1}(f.m.)] $\pm$ S.E.	
	24 h	48 h
Control	1.25 $\pm$ 0.32 (100)	1.46 $\pm$ 0.23 (100)
Salicylic acid	1.71 $\pm$ 0.14 (137)	1.97 $\pm$ 0.18 (135)
Caffeic acid	1.36 $\pm$ 0.12 (109)	1.86 $\pm$ 0.25 (127)
Gallic acid	1.01 $\pm$ 0.14 (81)	1.36 $\pm$ 0.22 (93)

Seeds were soaked in different phenolic acids, (0.1 mM) for either 24 or 48 h in light before planting. Seedlings were raised from these seeds, in distilled water for four days (including imbibition period). Values relative to control are given in brackets.

TABLE 3
Effect of aromatic amino acids in presence and absence of phenolic acids on anthocyanin content

Treatment	Anthocyanin content [A g^{-1}(f.m.)] ± S.E.
Control (Distilled water)	1.39 ± 0.06 (100)
Tyrosine (5 mM)	1.22 ± 0.13 (88)
Tyrosine + Salicylic acid	1.17 ± 0.09 (84)
Tyrosine + Caffeic acid	1.58 ± 0.16 (144)
Tyrosine + Gallic acid	1.63 ± 0.08 (117)
Phenylalanine (5 mM)	1.48 ± 0.23 (106)
Phenylalanine + Salicylic acid	2.34 ± 0.13 (168)
Phenylalanine + Caffeic acid	1.34 ± 0.07 (96)
Phenylalanine + Gallic acid	1.79 ± 0.07 (129)

Seedlings were raised either in amino acids alone or in combination with phenolic acids for four days. Values relative to control are given in brackets.

increasing the concentration of the acid between 0.01 and 5.0 mM, antho cyanin content also increased (Table 1). The magnitude of the effect of salicylic acid or gallic acid, however, remained unchanged due to increase in their concentration, when seeds were soaked in different phenolics (0.1 mM each) for either 24 or 48 h and then grown in distilled water the anthocyanin content was increased again with salicylic acid and caffeic acid but not with gallic acid treatment (Table 2). The difference in the effects of gallic acid during two types of treatment (supply during seedling growth or imbibition) may be a reflection of differences in its uptake and metabolism under two conditions. In our earlier observations with maize seedlings, 0.1 mM salicylic acid increased seedling growth, nitrogen content and enzymes of nitrogen assimilation pathway also (JAIN and SRIVASTAVA 1981a, b, c). Increase in anthocyanin and chlorophyll contents in maize by salicylic acid has been observed earlier by KHURANA and MAHESHWARI (1980) and MISHRA and SRIVASTAVA (1983), respectively. However, abscisic acid is known to decrease the anthocyanin level in maize roots (PILET and TAKAHASHI 1980). Further, cytokinin stimulated betacyanin synthesis in *Amaranthus caudatus* is inhibited by phenolic acids (CHALLICE 1977).

TABLE 4
Effect of glucose in the presence or absence of salicylic acid on anthocyanin content

Conc. of glucose [mM]	Anthocyanin content [A g^{-1}(f.m.)] ± S.E.	
	− salicylic acid	+ salicylic acid
0.00	1.39 ± 0.06 (100)	1.95 ± 0.02 (100)
1.00	1.92 ± 0.18 (138)	2.63 ± 0.32 (135)
5.00	1.97 ± 0.12 (142)	2.76 ± 0.07 (141)
10.00	2.27 ± 0.12 (163)	1.76 ± 0.09 (90)
20.00	2.72 ± 0.24 (196)	2.08 ± 0.41 (107)

Seedlings were raised in desired concentrations of glucose alone or in combination with 0.1 mM salicylic acid for four days. Values relative to control are given in brackets.

TABLE 5

Effect of different concentrations of shikimic acid in the presence or absence of salicylic acid on anthocyanin content

Conc. of shikimic acid[mM]	Anthocyanin content [A g^{-1}(f.m.)] ± S.E.	
	− salicylic acid	+ salicylic acid
0.00	1.39 ± 0.06 (100)	1.95 ± 0.02 (100)
0.01	2.77 ± 0.10 (199)	3.05 ± 0.31 (156)
0.10	2.76 ± 0.12 (199)	2.87 ± 0.23 (147)
1.00	2.79 ± 0.14 (201)	3.07 ± 0.14 (157)
5.00	2.83 ± 0.17 (204)	3.15 ± 0.16 (161)

Seedlings were raised in desired concentrations of shikimic acid alone or in combination with 0.1 mM salicylic acid for four days. Values relative to control are given in brackets.

To study the possible mechanism of action of phenolic acids on anthocyanin synthesis, aromatic amino acids tyrosine and phenylalanine, the possible precursors of anthocyanin and other flavonoids were supplied either alone or in combination with phenolic acids. While tyrosine had no effect, phenylalanine in combination with salicylic acid increased the anthocyanin content of maize roots (Table 3).

Addition of glucose, either alone or in combination with phenolic acid increased anthocyanin content (Table 4). MARGNA *et al.* (1974) observed an increase in anthocyanin content in radish seedlings by glucose, GREBINSKIĬ and KHMIL (1980) also observed the intensified synthesis of anthocyanins by addition of glucose with chlorocholine chloride in cabbage and spiderwort leaf discs. In the absence of salicylic acid the increase in anthocyanin content with glucose is concentration dependent while on addition of salicylic acid it increased at 1 and 5 mM and decreased at 10, and 20 mM. Supply of different concentrations of shikimic acid (Table 5) increased the anthocyanin content at all the concentrations alone and in combination with salicylic acid but the increase was almost equal at all the concentrations. The experiments do not support the possibility of salicylic acid or its metabolic products working as a precursor of anthocyanin synthesis. Possibly the effect of salicylic acid and other phenolic acids is indirect and more generalised. More detailed investigation is required to elucidate the role of phenolic acids in anthocyanin synthesis.

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BOOK REVIEW

GLUZMAN, Y., SHENK, T. (ed.): ENHANCERS AND EUKARYOTIC GENE EXPRESSION. — Cold Spring Harbor Laboratory, 1983. 218 pp. Paperback US $ 27.— in USA / $ 32.40 outside.

Transcription initiation represents an early and critical event in gene expression. As such, it is frequently the target of genetic regulatory mechanisms related to cellular differentiation and development, and adaptation to environmental conditions. Enhancers and regulatory elements is a relatively novel class of eukaryotic controlling DNA sequences that are capable of enhancing or activating gene transcription. They regulate the function of specific AT-rich DNA sequences called promoters which take part of inducible eukaryotic genes. The enhancer elements were originally discovered in SV 40 animal virus and were found to increase the transcriptional activity of the SV 40 promoter as well as of a variety of heterologous eukaryotic promoters. Functionally analogous regulatory sequences were later described in other animal viruses and also in genomic DNA of eukaryotic cells. The present book is devoted to the most recent advances in this field. It summarizes in the form of extended abstracts 36 contributions presented at a Conference on enhancers and eukaryotic gene expression held at the Banbury Center of the Cold Spring Harbor Laboratory on April 3—6, 1983. These abstracts include the majority of the known enhancer sequences as well as a large number of inducible promoters from virus, yeast, *Drosophila*. mouse, and human origin, and describe various experimental systems based on gene transfer and *in vitro* transcription methods enabling the DNA sequence of a molecule to be correlated with its transcriptional activity. A comparison of structure and function of enhancers and regulatory regions of different eukaryotic genes has revealed that whereas the enhancers are clearly separable from the corresponding promoters, the regulatory regions are usually closely associated with them. The book appeared as a title in the series of Current Communications in Molecular Biology and will be certainly appreciated especially by interested research workers as a condensed overview of the current more and more ingenious work in the rapidly progressing field of molecular mechanisms of transcription regulation.

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