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Effect of Some Growth Substances and Phenolic Compounds on Membrane Permeability in Beet Root

By

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Summary

BHANDAL I. S., SINGH R. & MALIK C. P. 1985. Effect of some growth substances and phenolic compounds on membrane permeability in beet root. — Phyton (Austria) 25 (1): 177—184, with 2 figures. — English with German summary.

Betacyanin efflux and conductivity changes were used as parameters of permeability in discs of beet root (*Beta vulgaris* L.). Among kinetin (Kn), gibberellic acid (GA_3), indole acetic acid (IAA), (2-chloroethyl)-phosphonic acid (Ethephon) and abscisic acid (ABA) only Kn and Ethephon promoted the pigment efflux and conductivity. Kn mediated response was faster one being discernible between 15—30 min. A concentration dependent nonspecific synergism was also recorded between Kn and other growth substances. Only conductivity was enhanced with cycocel and the other retardants, maleic hydrazide and 2,3,5-tri-iodobenzoic acid (TIBA), had no effect. Out of the phenols (α-naphthol, β-naphthol, salicylic acid and resorcinol) only salicylic acid enhanced pigment efflux and conductivity. While quercetin promoted the conductivity, no response was observed with rutin. It is inferred that betacyanin efflux can not serve as a sole criterion for membrane permeability. Furthermore, different substances differ in their mechanism of action vis-a-vis membrane permeability. Reduction of kinetin, Ethephon and salicylic acid effect at pH 7 suggests involvement of group(s) at the active site having their pK in this vicinity.

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Zusammenfassung

Bhadal I. S., Singh H. & Malik C. P. 1985. Wirkung einiger Wuchsstoffe und Phenolverbindungen auf die Membranpermeabilität der Roten Rübe. — Phyton (Austria) 25 (1): 177—184, mit 2 Abbildungen. — Englisch mit deutscher Zusammenfassung.

Der Austritt von Betacyanin und Änderungen der elektrolytischen Leitfähigkeit wurden als Parameter der Permeabilität der Zellen der Roten Rübe (*Beta vulgaris* L.) benutzt. Unter den geprüften Wuchsstoffen Kinetin (Kn), Gibberellinsäure (GA_3), Indolessigsäure (IAA), (2-Chloroethyl)-phosphonsäure (Etephon) und Abscisinsäure (ABA) beschleunigten nur Kn und Etephon den Pigmentaustritt und die Leitfähigkeit. Die Kn-Wirkung war rascher und innerhalb 15—30 min erkennbar. Zwischen Kinetin und anderen Wuchsstoffen wurde ein konzentrationsabhängiger unspezifischer Synergismus beobachtet. Cycocel erhöhte nur die Leitfähigkeit und die anderen Hemmstoffe (Maleinsäure und TIBA) waren wirkungslos. Außer den Phenolen (α- und β-Naphthol, Salicylsäure und Resorzin erhöhte nur Sylicylsäure Pigmentefflux und Leitfähigkeit. Quercetin erhöhte die Leitfähigkeit, nicht aber Rutin. Daraus wird gefolgert, daß der Betacyanaustritt nicht als alleiniges Kriterium für die Membranpermeabilität dienen kann, weiters daß sich die einzelnen Substanzen in ihrem Wirkungsmechanismus auf die Membranen unterscheiden. Bei pH 7 ist die Wirkung von Kinetin, Ethephon und Salicylsäure herabgesetzt, was die Beteiligung von aktiven Gruppen mit einem pK in diesem Bereich nahelegt.

(Editor transl.)

1. Introduction

Betacyanin efflux from beet root discs has been widely used as am permeability marker (Gäumann, Jaag & Braun 1947, Gäumann & v. Arx 1947, Siegel & Halpern 1964, 1965, Siegel 1969, 1970, Grunwald 1970, Naik & Srivastava 1981, Fabijan & al. 1981, Naik & al. 1980. Among the factors which are reported to enhance efflux are alcohols, polycations, acids, temperature, inorganic salts, polyamines and some growth substances. Some of these studies were carried out at higher temperature (37° C) which itself leads to considerable efflux (Naik & Srivastava 1981; Naik & al. 1980). Secondly, most of these studies used only one permeability parameter i. e. betacyanin efflux which possibly involves large pores, betacyanin being a large molecule (Dreiding 1961). The present investigation, thus, involves studies at low temperature where two permeability parameters used were betacyanin, efflux and conductivity so as to obtain precise results vis-a-vis membrane permeability.

2. Materials and Methods

Beet root (*Beta vulgaris* L.) was used as an experimental material in this investigation where betacyanin efflux and conductivity changes were taken as the two parameters of permeability. Discs of 3 mm thickness and 11 mm in diameter were prepared and washed in running water till there was no further significant pigment efflux. After drying up 10 discs were weighed and incubated in 10 ml of test solutions up to 3 h at 28 ± 2° C whereupon conductivity and absorbance A at 535 nm were recorded. The data were, however, calculated on the basis of $g^{-1} \cdot 10\ ml^{-1}$ for comparison of the results. Stock solutions of indoleacetic acid gibberellic acid, abscisic acid, maleic hydrazide and phenols were prepared by dissolving the weighed substance in a few drops of ethanol and then the required volume was made. In case of 2, 3, 5-tri-iodobenzoic acid methanol was used instead. Kinetin, on the other hand, was solublised in a few drops of 0.5 N HCl. Ethephone and cycocel are available in liquid form. In all cases, pH of the solutions was adjusted to 7.

3. Results

3.1. Growth substances

Among the different growth substances (Kn, IAA, GA_3, Ethephon and ABA) tested at various concentrations (0.1, 0.5, 1, 5, 10, 25 and 50 ppm) only Kn and Ethephon promoted betacyanin efflux (Table 1).

Table 1

Effect of different concentrations of Kn, GA_3, IAA, Ethephon and ABA on betacyanin efflux (A_{535}, $g^{-1} \cdot 10\ ml^{-1}$) measured after 3 h. Betacyanin efflux in distilled water control was 0.09

Concentration (ppm)	Growth substance				
	Kn	GA_3	IAA	Ethephon	ABA
0.1	0.11	0.07	0.10	0.10	0.05
0.5	0.13	0.10	0.12	0.11	0.05
1	0.09	0.09	0.12	0.09	0.04
5	0.78*	0.07	0.15	0.08	0.06
10	3.4*	0.10	0.15	0.09	0.03
25	4.8*	0.07	0.15	0.09	0.08
50	4.4*	0.09	0.14	0.47*	0.03

* Significant at 5% level

The Kn effect begins at 5 ppm and somewhat stabilizes between 25 and 50 ppm concentrations. Only higher concentration (50 ppm) has promotory effect in case of Ethephon treatment. Kn response though observable after 15 min of incubation, yet becomes significant only between 15 to 30 min interval (Fig. 1). Kn effect on betacyanin efflux is also

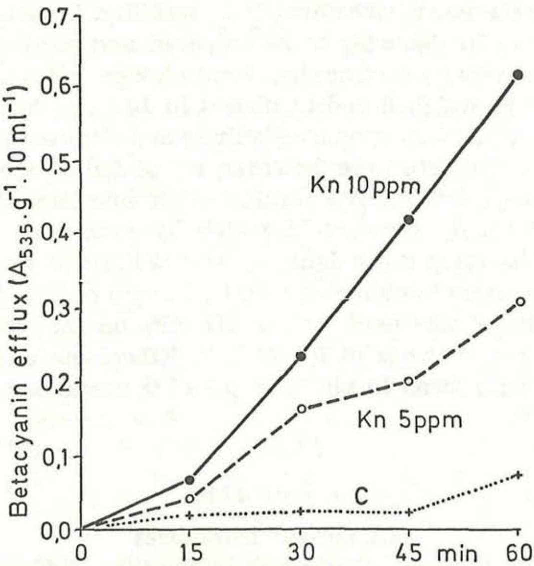

Fig. 1. Effect of kinetin (Kn 5 ppm and 10 ppm) on betacyanin efflux. C = Control

accompanied by enhanced conductivity (Table 2 & 3). GA_3, IAA, Ethephon and ABA evoke pigment efflux when used in combination with Kn at 10 ppm concentration (Table 3). Such combinations at 5 ppm, however, did not elicit any effect (Table 2).

Table 2

Effect of Kn treatment along with GA_3, IAA, Ethephon and ABA on betacyanin efflux and conductance recorded after 3 h. The concentration was 5 ppm each

Treatment	Betacyanin efflux A_{535}, $g^{-1} \cdot 10\ ml^{-1}$	Conductance mhos, $g^{-1} \cdot 10\ ml^{-1}$
Control	0.02	0.13
Kn	0.30*	0.84*
GA_3	0.09	0.12
IAA	0.08	0.14
Ethephon	0.05	0.12
ABA	0.09	0.13
Kn + GA_3	0.19°	0.76°
Kn + IAA	0.26°	0.78°
Kn + Ethephon	0.24°	0.74°
Kn + ABA	0.21°	0.76°

* Significant at 5% level of significance with respect to control
° Non-significant at 5% level of significance with respect to Kn

Table 3

Effect of Kn treatment along with GA_3, IAA, Ethephon and ABA on betacyanin efflux and conductance measured after 3 h. The concentration was 10 ppm each

Treatment	Betacyanin efflux A_{535}, $g^{-1} \cdot 10\ ml^{-1}$	Conductance mhos, $g^{-1} \cdot 10\ ml^{-1}$
Control	0.04	0.56
Kn	0.54*	0.98*
GA_3	0.05	0.58
IAA	0.08	0.58
Ethephon	0.10	0.62
ABA	0.08	0.66
Kn + GA_3	0.78+	1.06
Kn + IAA	0.84+	1.08
Kn + Ethephon	0.80+	1.12
Kn + ABA	0.82+	0.99

* Significant at 5% level of significance with respect to control
\+ Significant at 5% level of significance with respect to Kn

3.2 Growth retardants

It is evident from table 4 that the three retardants; antiauxins (maleic hydrazide and TIBA) and antigibberellin (cycocel), did not effect betacyanin efflux at any concentration (5, 10 and 20 ppm). However, cycocel increased the conductivity at higher concentration (Table 4).

Table 4

Effect of cycocel, maleic hydrazide and 2, 3, 5-tri-iodobenzoic acid on betacyanin efflux and conductance measured after 3 h

Treatment	Concentration (ppm)	Betacyanin efflux (A 535 gm^{-1} 10 ml^{-1})	Conductance (mhos, gm^{-1} 10 ml^{-1})
Control	–	0.10	0.10
Cycocel	5	0.16	0.10
Cycocel	10	0.12	0.26*
Cycocel	20	0.13	0.37*
Maleic hydrazide	5	0.11	0.11
Maleic hydrazide	10	0.07	0.09
Maleic hydrazide	20	0.11	0.08
2, 3, 5-tri-iodobenzoic acid	5	0.00	0.06
2, 3, 5-tri-iodobenzoic acid	10	0.00	0.06
2, 3, 5-tri-iodobenzoic acid	20	0.00	0.07

* Significant at 5% level of significance

3.3. Phenolic substances

Effect of four phenols (α-naphthol, β-naphthol, resorcinol and salicylic acid) and two flavonoids (rutin and quercetin) was investigated using 0.1, 1, 5, 10, 25 and 50 ppm concentrations. No betacyanin efflux was observed except in case of salicylic acid where 25 and 50 ppm concentrations enhanced betacyanin efflux (0.17 and 0.59 respectively) and conductivity. Quercetin, on the other hand, increased the conductivity only (Table 5).

Table 5

Effect of different concentrations of α-naphthol, β-naphthol, resorcinol, salicylic acid, rutin and quercetin on conductance (mhos $g^{-1} \cdot 10\ ml^{-1}$) measured after 3 h. The conductance in distilled water was 0.14

Treatment	Concentration (ppm)					
	0.5	1	5	10	25	50
α-naphthol	0.10	0.18	0.18	0.17	0.18	0.20
β-naphthol	0.14	0.14	0.14	0.14	0.16	0.17
Resorcinol	0.05	0.04	0.05	0.05	0.07	0.05
Salicylic acid	0.16	0.17	0.16	0.16	0.19	0.29*
Rutin	0.08	0.07	0.09	0.09	0.09	0.10
Quercetin	0.12	0.04	0.15	0.16	0.27*	0.37*

* Significant at 5% level of significance

3.4 Effect of pH

Fig. 2 shows reduction in betacyanin efflux when Kn, Ethephon and salicylic acid solutions were prepared in tris-HCl or phosphate buffer (0.1 M, pH 7).

4. Discussion

Naik & al. (1980) studied the effect of kinetin, GA_3, Ethephon and 2,4-D on betacyanin efflux where only Kn (1 mM) was shown to be promotory. Our investigations though support their observations yet are different since observations were recorded at 28 ± 2° C instead of 37° C, where higher temperature itself promotes the efflux. Temporal study of kinetin effect suggests Kn response to be a rapid one. Ethephon evoked response appears only at higher concentration (50 ppm). GA_3, IAA and ABA individually did not affect any significant change in permeability though Naik & al. (1980) have reported reduction in betacyanin efflux with GA_3 and Ethephon at 37° C. A concentration dependent synergism was observed between Kn and other growth substances which appears to be non-selective.

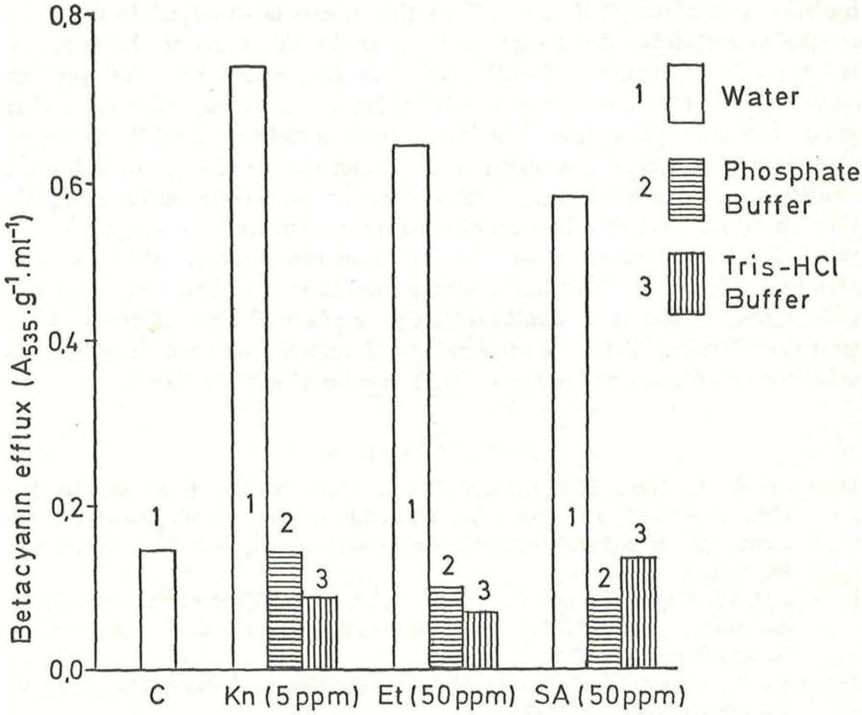

Fig. 2. Effect of kinetin (Kn, 5 ppm), 2-chloroethylphosphonic acid (Ethephon, Et, 50 ppm) and salicylic acid (SA, 50 ppm) on betacyanin efflux when supplemented in water (1), 0.1 M phosphate buffer pH 7 (2) and 0.1 M tris-HCl-buffer pH 7 (3). C = Control

Contrary to the reports of Fabijan & al. (1981) where higher efflux with antigibberellin (Cycocel and Amo 1968) has been reported we failed to notice any pigment efflux with antigibberellin (cycocel) or antiauxins (TIBA and maleic hydrazide). Perhaps the concentration used in earlier reports was too high (5×10^{-3}M).

Among the phenols and phenolic substances tested, only salicylic acid promoted the betacyanin efflux. Harper & Balke (1979) have reported an inhibition of ion absorption in excised oat roots with salicylic acid. The differential effect of salicylic acid on these two permeability parameters is understandable in terms of the different nature of ion absorption (active) and betacyanin efflux (passive). Grunwald (1970) has shown that betacyanin efflux was independent of respiration. Alternatively, salicylic acid response may be an acid response only. Siegel (1970) has already reported enhanced pigment efflux under acidic condition.

The effectors viz. kinetin, Ethephon and salicylic acid caused reduced effect in presence of PO_4^{-3} and tris-HCl buffer at pH 7. Perhaps ionization of group(s) having pK in this range is involved in the permeability response. The nature of these molecules can only be speculated though importance of -SH groups in membrane organization has been realized (Naik & Srivastava 1981, Naik & al. 1980). However, -SH group has high pk value. It is interesting to note that while betacyanin efflux is always accompanied by conductance change of solution, results are also available (cycocel and quercetin) where only conductivity changes. Perhaps, betacyanin efflux is affected by large passive pores for large molecules so that ions and other small molecules can also leak. However, there also seems a second mechanism where permeability only to ions and small molecules is affected, the different effectors thus having different mechanism of action. Betacyanin efflux itself thus should not be used as a sole criterion of permeability.

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