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BRIEF COMMUNICATION

The response of nitrate reductase activity and nitrate assimilation in maize roots to growth regulators at acidic pH

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Abstract

Nitrate and total nitrogen contents, and nitrate reductase (NR) activity of the excised maize roots in buffered or unbuffered nitrate solution (at pH 6.5 or 4.5) as affected by putrescine (PUT), abscisic acid (ABA) and salicylic acid (SA) were investigated. In unbufferred solution, the NR activity was lower at pH 4.5 as compared to that at pH 6.5, but in bufferred solution the activity was higher at lower pH. Supply of 100 μM PUT or 500 μM SA, promoted NR activity and 50 μM ABA inhibited the activity at pH 6.5. However, at pH 4.5, PUT and SA inhibited NR activity and ABA had no effect. In most cases, the increase in NR activity was positively correlated with total organic nitrogen and a negatively with nitrate content. A reverse situation was found when NR activity was inhibited by the growth regulators.

Additional key words: abscisic acid, nitrate content, putrescine, salicylic acid.

The effect of nutrient solution acidity on nitrate reductase activity (NR, E.C. 1.6.6.1-2) and nitrate assimilation is well known. The NR activity in the roots and shoots of maize was higher when the seedlings were raised at pH 6.3 than at pH 4.3 (Sinha and Srivastava 1998). However, when the excised roots were incubated in a Tris buffer solution containing nitrate, NR activity was lower at pH 6.5 than at 4.5 (Shankar *et al.* 2000). In other relevant studies, anaerobiosis, mannose feeding, or addition of permeating weak acids, increased NR activity (Kaiser *et al.* 1999). Many other physiological and metabolic effects of acidic pH on plants are also known and the external pH is now considered to be an important environmental signal. Like many other environmental factors, the effect of pH may also be mediated via other signals or messengers. For example, the intracellular pH variations caused by some environmental stresses seem to be acting on leaf elongation rate through ABA (Wilkinson and Davies 1997, Bacon *et al.* 1998). Polyamines act as buffers during ammonia assimilation (Altman and Levin 1993). It is also known that the low external pH causes accumulation of putrescine (PUT), which in turn tries to maintain the internal pH (Smith and Sinclair 1967, Young and Galston 1983). Abscisic acid (ABA), polyamines, and salicylic acid (SA) are also known to affect the NR activity and nitrate assimilation in plants (Jaiwal and Singh 1995, Pandey and Srivastava 1995) although it is not known, whether their effects varied according to the environmental pH. This aspect has been investigated in the present study.

Seeds of *Zea mays* L. cv. Ganga safed-2 were sterilized for 5 min with 5 % solution of $CaOCl_2$ and then washed thoroughly with distilled water. Then they were planted on double layer of filter paper moistened with 1/2 strength modified Hoagland's solution (pH 6.5) containing no nitrogen in 15-cm Petri dishes (50 seeds per dish). Seedlings were raised at 12-h photoperiod with irradiance of 70 W m^{-2} and temperature 25 ± 2 °C for 5 - 6 d. The excised (5 - 6 mm long) root segments were incubated in either unbufferred (aqueous) or bufferred (0.1 M Tris buffer) 5 mM KNO_3 solution of pH 6.5 or 4.5 for 24 h at 27 ± 2 °C under irradiance of 70 W m^{-2}. Three concentrations of the growth regulators were used: PUT - 20, 100, 500 μM; ABA - l, 10, 50 μM, and SA - 20, 100,

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Abbreviations: ABA - abscisic acid; NR - nitrate reductase; PUT - putrescine; SA - salicylic acid.

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500 μM. However, only the data for 100 μM PUT, 50 μM ABA, and 500 μM SA are given in this paper because most parameters were significantly affected at these concentrations.

Nitrate reductase (NADH specific) activity in the freshly harvested root samples was measured by *in vitro* method (Srivastava and Ormrod 1984). For measuring total nitrate and Kjeldahl nitrogen content in oven dried (65 °C for 48 h) root samples the methods of Catoldo *et al.* (1975) and of Lang (1958), respectively, were followed.

As reported earlier (Shankar *et al.* 2000), *in vitro* NADH:NR activity in the excised roots incubated for 24 h in 5 mM KNO_3 was lower at pH 4.5 than at pH 6.5 in the unbufferred solution, although the effect of pH was reversed in bufferred solution, *i.e.* in buffered solution, the enzyme activity was higher at pH 4.5 than at pH 6.5 (Table 1). Experiments also indicated that the effect of growth regulators PUT, ABA and SA on NR activity was influenced by the medium pH and the buffering potential of the medium. In comparison with control, 100 μM PUT and 500 μM SA increased NR activity at pH 6.5 and decreased it slightly at pH 4.5 in both bufferred and unbufferred solutions (Table 1). ABA (50 μM) however, caused a decline in NR activity. In earlier studies, PUT and SA increased NR activity at near or at neutral pH (Jain and Srivastava 1981, Pandey and Srivastava 1995) and ABA decreased it (Jaiwal and Singh 1995).

Table 1. Effect of 100 μM putrescine, 50 μM abscisic acid, or 500 μM salicylic acid on nitrate reductase activity [nmol(nitrite) g^{-1}(f.m.) s^{-1}] in excised maize roots incubated at pH 6.5 or 4.5 in bufferred or unbufferred solutions for 24 h. Means ± SE, $n = 6$.

	Unbufferred		Bufferred	
	6.5	4.5	6.5	4.5
Control	0.87 ± 0.08	0.74 ± 0.04	0.85 ± 0.06	1.16 ± 0.07
PUT	1.05 ± 0.14	0.68 ± 0.00	0.99 ± 0.06	1.22 ± 0.08
ABA	0.48 ± 0.04	0.79 ± 0.05	0.60 ± 0.05	1.24 ± 0.12
SA	1.10 ± 0.11	0.40 ± 0.03	1.52 ± 0.15	0.66 ± 0.05

Table 2. Increase in pH of the incubation medium during incubation of excised roots in the presence of 100 μM putrescine, 50 μM abscisic acid, or 500 μM salicylic acid. Initial pH was 6.5 or 4.5. Means ± SE, $n = 6$.

	Unbufferred		Bufferred	
	6.5	4.5	6.5	4.5
Control	1.4 ± 0.10	1.8 ± 0.09	0.0 ± 0.00	1.5 ± 0.12
PUT	0.8 ± 0.03	1.8 ± 0.12	0.1 ± 0.00	1.4 ± 0.07
ABA	1.2 ± 0.10	3.0 ± 0.19	0.1 ± 0.00	0.2 ± 0.00
SA	0.8 ± 0.04	0.9 ± 0.01	0.2 ± 0.00	1.5 ± 0.07

The medium pH increased slightly during 24 h incubation of excised roots (Table 2). PUT had little effects on increase in pH. ABA either elevated (unbufferred) or reduced (bufferred) the increase in pH at 4.5 pH and SA reduced the increase in pH in unbufferred solution. The effects of growth regulators on differences in pH were correlated with their effects on NR activity. In most cases, the increase in NR activity was positively correlated with increase in total organic nitrogen and a decrease in NO_3^- content. For example, PUT caused a decline in nitrate content in unbufferred solutions but increased it slightly in bufferred solutions (Table 3), and had little effect on total nitrogen content at 4.5 pH and increased it at pH 6.5 (Table 4). ABA reduced nitrate and total nitrogen contents at pH 6.5 but had almost no effect at 4.5 pH in both bufferred as well as unbufferred solutions. SA declined nitrate content in all treatments (Table 3) and increased total nitrogen at pH 6.5 and declined it at pH 4.5 (Table 4).

Table 3. Effect of 100 μM putrescine, 50 μM abscisic acid, or 500 μM salicylic acid on nitrate content [mg g^{-1}(d.m.)] of the roots at pH 6.5 or 4.5. Means ± SE, $n = 6$.

	Unbufferred		Bufferred	
	6.5	4.5	6.5	4.5
Control	4.1 ± 3.9	25.5 ± 2.9	42.2 ± 0.32	27.2 ± 0.36
PUT	21.0 ± 2.6	20.5 ± 2.2	45.6 ± 0.34	29.2 ± 0.31
ABA	36.5 ± 3.1	25.5 ± 2.3	34.1 ± 0.21	26.2 ± 0.33
SA	10.8 ± 0.6	14.1 ± 1.6	20.2 ± 0.22	19.3 ± 0.24

Table 4. Effect of 100 μM putrescine, 50 μM abscisic acid, or 500 μM salicylic acid on total nitrogen content [mg g^{-1}(d.m.)] of the roots at pH 6.5 or 4.5. Means ± SE, $n = 6$.

	Unbufferred		Bufferred	
	6.5	4.5	6.5	4.5
Control	32.4 ± 2.3	41.0 ± 3.5	30.2 ± 0.41	36.0 ± 0.29
PUT	36.4 ± 1.9	39.4 ± 2.2	37.9 ± 0.33	34.9 ± 0.28
ABA	29.0 ± 3.1	36.3 ± 1.2	24.4 ± 0.18	29.6 ± 0.33
SA	48.0 ± 1.7	22.5 ± 2.5	38.2 ± 0.12	33.6 ± 0.37

It is possible to provide some explanation for the pH dependent effects of growth regulators on NR activity in some cases. PUT increased enzyme activity at pH 6.5, but it was either inhibitory or no effect at pH 4.5, apparently because low pH caused accumulation of endogenous PUT (Young and Galston 1983), and hence external PUT had no effect. An increase in cytoplasmic pH in response to applied abscisic acid has been reported in maize hypocotyl cells (Gehring *et al.* 1990) and in barley aleurone protoplasts (Van der Veen *et al.* 1992). This increase in internal pH appears to be necessary for ABA

induced gene expression (Vander Veen *et al.* 1992). The differential responses to salicylic acid at pH 6.5 and 4.5 may be explained to some extent on the basis of the different rates of uptake of salicylic acid from the medium at two pH. Khurana and Cleland (1992) demonstrated that uptake of SA by *Lemna paucicostata* was significantly higher at pH 4.5 than 6.5.

Although these experiments do not provide any direct evidence for the possible role of growth regulators as a secondary messengers for low pH, their variable effects on NR activity according to pH and the buffering potential of the medium indicate some interactions.

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