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Growth and mineral composition of grape-vine rootstock cultured *in vitro* with different levels of ammonium nitrate

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Abstract

Growth and mineral composition of grape-vine explants (13.3 EVEX rootstock) cultured on a basal medium with 10.4 m*M* of NO_3^-, and respective additions of 5, 10, 15, 20 and 25 m*M* of NH_4NO_3 were analysed.

Low N-availability (10.4 m*M*) induced low shoot formation. Addition of NH_4NO_3 up to 15 m*M* increased number, quality and N levels of shoots. Further increase of N in the medium induced very high contents of N and water in tissues, and led to a bad shoot quality (large and abnormally shaped leaves and dark colour-fragile tissues, difficult to handle in further propagations). The content of K in explants was negatively affected by the concentration of NH_4NO_3 in the substrate and by the level of N in tissues.

Introduction

In a previous work (Troncoso *et al.*, 1988) the influence of N on growth and mineral composition of grape-vine rootstocks 41 B and 161–49, were studied. Although the response of the rootstock 161–49 was much more active than that of 41 B, increasing N in the medium increased number, size and N contents of shoots and decreased formation of callus in both rootstocks, but excess of N produced non-desired shoots.

The aim of the present work was to study the response to N of a new grape-vine rootstock (13.3 EVEX) from the "Estación de Viticultura y Enologa de Jerez (Spain)", and to study more in detail the influence of N availability on the quality and mineral composition of shoots.

Materials and methods

Homogeneous, 10 mm long explants of grape-vine rootstock 13.3 EVEX were cultured at a temperature of 25°C, light intensity of 2500 lux and 16 h photoperiod.

The treatments were:
—Control, with basal substrate (Table 1);
—Five NH_4NO_3 concentrations added to the

Table 1. Composition of the basal nutritive medium

Chemical compound	m*M*	Chemical compound	µM	Chemical compound	µM
KNO_3	7.91	$MnSO_4.4H_2O$	5.0	M-inositol	27.75
$Ca(NO_3)_2.4H_2O$	1.27	H_3BO_3	100.0	Thiamine	2.96
KH_2PO_4	1.25	$ZnSO_4.7H_2O$	30.0	6-BAP	4.43
$MgSO_4.7H_2O$	1.50	$Na_2MoO_4.2H_2O$	1.0	IBA	0.48
$FeSO_4.7H_2O$	0.09	$CuSO_4.5H_2O$	0.1	Sucrose	30 g.L^{-1}
Na_2EDTA	0.10	$CoCl_2.6H_2O$	0.1	Agar	6 g.L^{-1}

Table 2. Influence of NH_4NO_3 on number, growth, hydration and mineral composition of shoots

NH_4NO_3 added (m*M*)	Shoots per explant (average)	% of shoots >10 mm	Fresh weight (mg/plant)	% hydration	Mineral composition		
					N (%)	K (%)	Fe (mg/kg)
0	2.60a	25.93a	125	81.25	1.32	2.95	85
5	4.01b	43.09b	220	82.50	2.54	2.52	161
10	5.76b	43.84b	345	82.92	3.39	2.20	234
15	5.00b	45.84b	539	83.23	4.03	1.77	336
20	5.37b	51.19b	541	85.50	4.79	1.49	431
25	5.49b	49.74b	499	89.75	5.50	1.48	530

Identical letters indicate non-significant difference ($P < 0.05$) by the Tukey test.

control medium: 5, 10, 15, 20 and 25 m*M* of the salt, respectively.

Each treatment involved 24 explants (8×3 replicates).

After 30 days, the number and size (per cent under and over 10 mm) of shoots, fresh and dry weight, tissue hydration (fresh weight minus dry weight/fresh weight $\times$ 100) and mineral composition (Pinta *et al.*, 1969; 1973) of explants were analysed. To determine the quality of shoots, size and shape of leaves, colour and fragility of tissues, and facility to handle in further propagation processes were also considered.

Results and discussion

As shown in Table 2, low concentration of N in the medium (control) produced low number and size of shoots. Additions of NH_4NO_3 up to 10 m*M* increased both number and length of shoots, and higher N additions did not cause further increase of these characteristics. The rootstock 13.3 EVEX showed a lower response to N than 161–49 and 41 B (Troncoso *et al.*, 1988).

Addition of NH_4NO_3 to the substrate up to 15 m*M*, resulted in a linear increase of shoot fresh weight ($r = 0.99$). Further increase in N addition had a negative effect on the weight of shoots. Addition of NH_4NO_3 resulted in increased hydration percentage ($r = 0.93$), N content ($r = 0.97$) and Fe content ($r = 0.99$) of explant tissues. In contrast, the levels of K in tissues were negatively affected by NH_4NO_3 additions ($r = -0.96$) and by N in tissues ($r = -0.99$). The other nutrients considered were not clearly influenced by N, their average levels being 3.3 g/kg for P, 5.8 g/kg for Ca, 3.0 g/kg for Mg, 43 mg/kg for Cu, 124 mg/kg for Mn, and 241 mg/kg for Zn, all on a dry weight basis.

N-applications clearly affected the quality of shoots. Low N availability produced few and short shoots. Addition of 10 or 15 m*M* of NH_4NO_3 gave more, larger, well-formed shoots, with normally sized-shaped leaves, intensive green colour and flexible tissues, very easy to handle and with good response in further propagations. Larger N-additions, 20 m*M* and more, produced non-desired shoots with very dark colour, abnormally large shaped leaves and fragile tissues, difficult to handle in further propagation processes.

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