

Effects of Thidiazuron on Grapevine Axillary Buds Cultivated in Vitro

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N-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea [thidiazuron (TDZ)], a newly available phenylurea, has been reported to have high cytokinin activity at low concentrations. Its cytokinin-like effects on several species include promotion of growth in cell and callus cultures, stimulation of in vitro shoot proliferation and somatic embryogenesis, and budbreak of dormant trees (Matsuda and Hirabayashi, 1989; Mok et al., 1987). The aim of this work is to test grapevine response to TDZ during in vitro culture of axillary buds.

Woody cuttings of *Vitis vinifera* L. 'Barbera' (clone CVT-AT84) were forced to sprout. After 6 weeks, the new shoots were collected and sterilized by immersion in a 25% (v/v) aqueous solution of a 7% chlorine detergent for 15 min. Explants, isolated from the sterilized shoots, were nodal segments formed by the axillary buds plus a small part of the adjacent tissues of stem and petiole (0.8 cm average length). Buds were cultured on an agarized Nitsch and Nitsch (1969) medium, modified by increasing thiamine HCl to 15 μM and FeEDTA to 200 μM, and containing 10, 1, 0.1, 0.01, 0.001, or 0 μM TDZ (Schering S.p.A., Segrate, Italy). The pH of the media was adjusted to 5.6 before autoclaving. Cultures were grown at 23C with a 16-h photoperiod and 50 μmol·s⁻¹·m⁻²

photosynthetic photon flux. The experiment was conducted twice, and the number of explants per treatment varied between 16 and 26, depending on the incidence of contamination.

After 1 month of culture, shoots (1 cm long) from unrooted nodal segments were excised and placed on a growth regulator-free medium (Nitsch and Nitsch, 1969, modified as described above).

TDZ was effective in promoting bud outgrowth (Table 1) and increasing the number of shoots obtained from a single bud. It must be noted, however, that grapevine usually shows a relatively low proliferation rate even in the presence of benzyladenine (BA) in the medium. In previous experiments with 'Barbera' on the same basal medium with 5 μM BA, about three shoot/nodal segments were obtained (unpublished results).

Some of the explants also produced roots from the basal part of the nodal segment, and at concentrations of TDZ above 0.1 μM, the

incidence of rooting was reduced or eliminated (Table 1). Symptoms of vitrification and leaf malformations, such as deeply lobed leaf blades, dentations, and presence of anthocyanins, became more pronounced as concentration of TDZ increased, with 0.1 μM or higher concentrations causing thickening of the stems. At the two highest doses, large amounts of callus were formed at the base of the explants. Shoot number and length decreased at the highest concentration.

After excision of shoots from the unrooted nodal segments and their transfer to a growth regulator-free medium, the first one to two newly formed leaves showed malformations when the shoots were grown previously on medium containing 1 or 10 μM TDZ. Percentage of shoot rooting ranged from 50% to 80%, except for shoots from 10 μM TDZ, which did not root. Some of the results presented here are similar to those reported for other species. On this clone of grape, low concentrations promote bud outgrowth and development of relatively healthy shoots, showing that TDZ could be used to multiply grapevine in vitro, but the negative effects of TDZ seem to be pronounced. Experiments on different grapevine cultivars are needed to confirm TDZ effectiveness.

Literature Cited

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Table 1. Effects of TDZ on proliferation and rooting of grapevine nodal segments after 1 month of culture in vitro. Morphological visual evaluation rating: from 0 (strongly vitrified and/or malformed shoots) to 4 (fully normal and healthy shoots).

TDZ concn (μM)	Sprouted explants (%)	Rooted explants (%)	No. shoots/explant	Shoot length (mm)	Morphological evaluation (0-4)
0	47	42	1.11	10.7	3.2
0.001	81	57	1.00	11.2	2.4
0.01	82	64	1.17	13.9	2.4
0.1	75	12	1.59	13.3	1.9
1	91	0	2.15	10.9	1.2
10	96	0	1.72	9.5	0.5
Source of variance					
Linear	*	*	*	*	**
Quadratic	NS	**	**	NS	**
Lack of fit	NS	NS	NS	NS	NS

NS,*,**Nonsignificant or significant at *P* = 0.05 or 0.01, respectively.

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