TDZ, 2iP and Zeatin in blueberry (*Vaccinium corymbosum* L. var. Duke) *in vitro* proliferation and organogenesis

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Introduction

Zeatin (Reed *et al*., 1991) and Benzyladenine (Tirone *et al.*, 2011) are the most important cytokinins used in commercial propagation of a large number of plants and also blueberry varieties. Due to the high costs of Zeatin several studies were aimed to test the efficiency of others growth regulators to induce proliferation and regeneration of different genotypes. TDZ resulted a really efficient alternative cytokinin-like compound for inducing leaf organogenesis in other soft fruit species like strawberry (Landi e Mezzetti, 2006) and for other biotecnological regeneration approach like promoting meristematic bulk, regeneration technique successfully used for other species like grape (Mezzetti *et al*., 2002).

In this study, TDZ, alone or in combination with 2iP, was tested for inducing high shoot proliferation, leaf tissue organogenesis, callus formation in in vitro blueberry (Cv. Duke). TDZ induced a high callus formation at both concentration (0,2 and 0,5 mg l^{-1}) tested, but if combined with 2iP (15 mg l^{-1}) callus formation was inhibited and stems elongation promoted. The control, a medium supplemented with 2 mg l^{-1} of Zeatin (an example of commercial proliferation media), showed high elongation of the stems and a reduced proliferation rate in comparison with TDZ.

TDZ can be considered an interesting PGR for improving blueberry in vitro proliferation and regeneration efficiency, however, further studies should be carried out in order to verify phenotypic and genotypic stability of the new plants obtained.

PROLIFERATION

Materials (1)

5 jars x 15 plants each x treatment

Media Used: WPM elements and vitamins + 30 g l^{-1} Sucrose, 7,5 Plant agar, pH=4,9

WPM1: without PGR (CT);

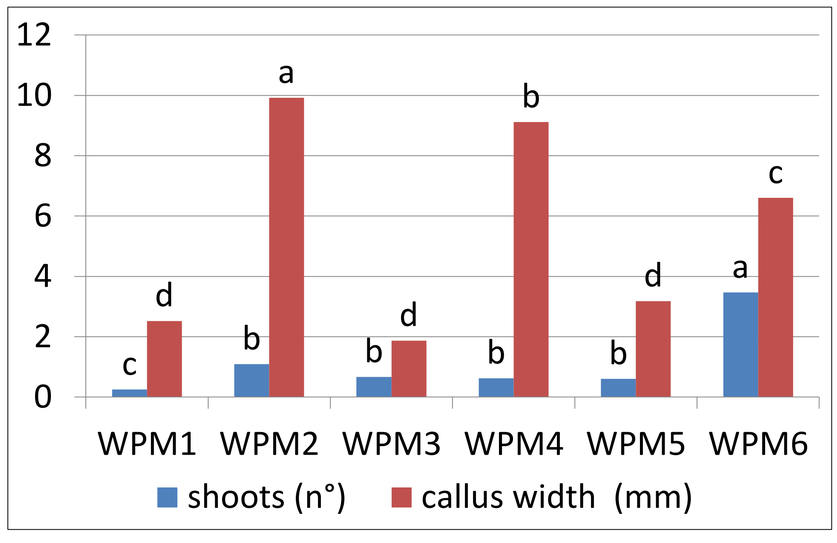
WPM2: + TDZ 0,2 mg l^{-1};

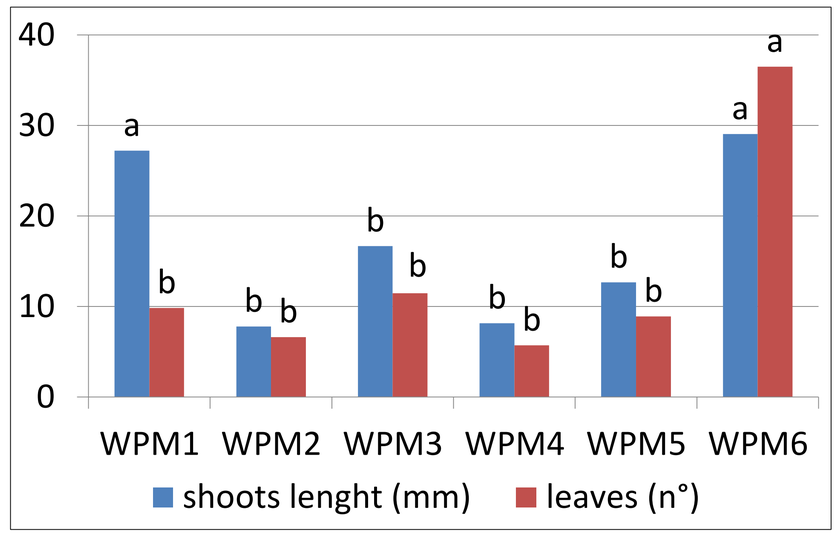
WPM3: + TDZ 0,2 mg l^{-1} + 2iP 15 mg l^{-1};

WPM4: + TDZ 0,5 mg l^{-1};

WPM5: + TDZ 0,5 mg l^{-1}+ 2iP 15 mg l^{-1};

WPM6: + Zeatin 2 mgl^{-1}.





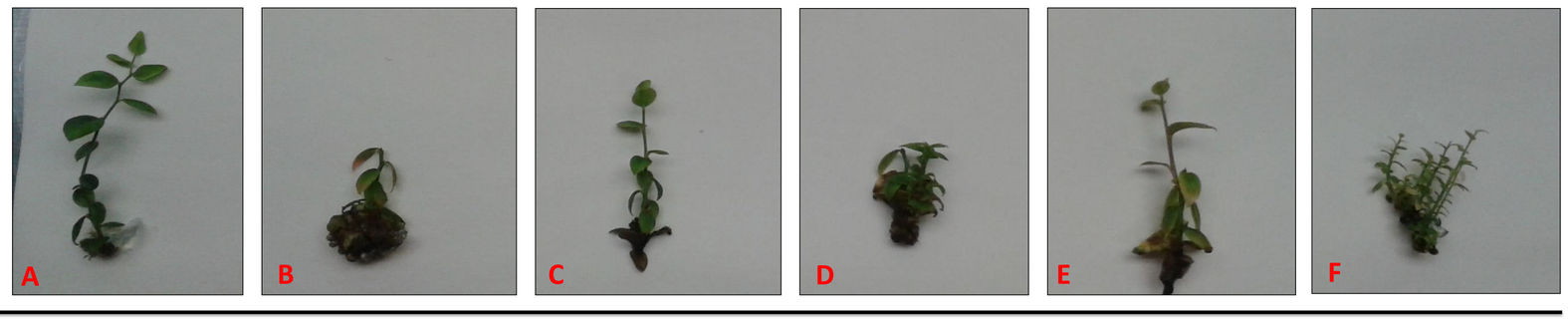
Results (1)

A= elongated shoots obtained in a media without cytokinins.

B & D= callus formation obtained with a media supplemented with TDZ (0,2-0,5 mg l^{-1})

C & E= callus inhibition by the presence of 2iP

F= axillary bud proliferation in a media supplemented with Zeatin (2 mg l^{-1})



DIRECT AND INDIRECT ORGANOGENESIS

In vitro proliferating shoots were used to develop two different regeneration approaches: A) leaves were cut perpendicularly to the central vein and transferred onto petri dish with the same media as before. B) callus obtained were divided in slice and transferred to a different media (M&M 2) in order to establish meristematic bulks.

Materials (2)

5 jars (or petri dish) x 15 slices or leaf x treatment

Media Used: WPM elements and vitamins + 30 g/l Sucrose, 7,5 Plant agar, pH=4,9

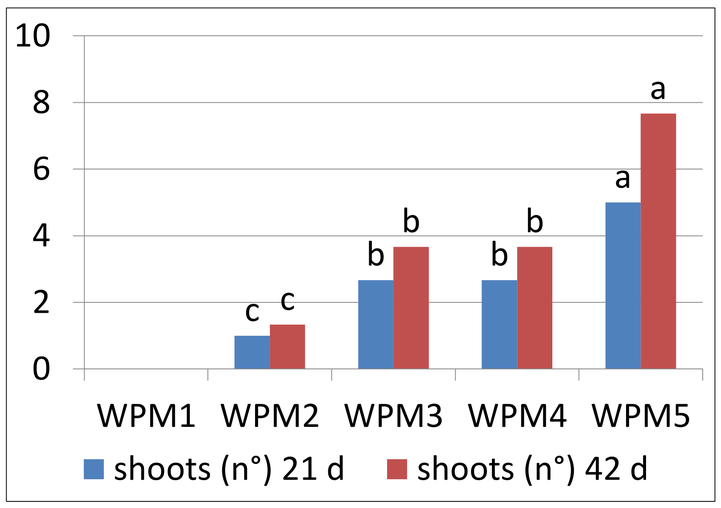
WPM1: without PGR (CT);

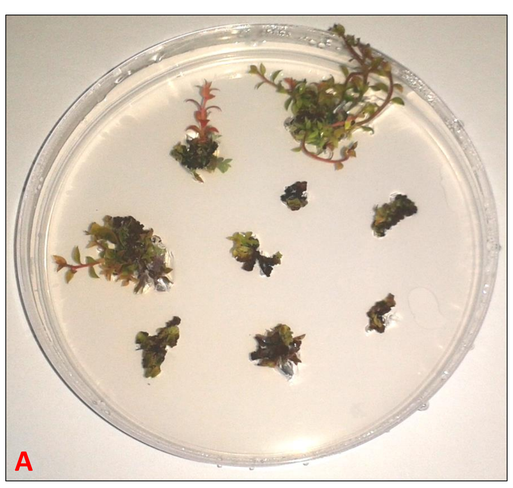
WPM2: + TDZ 0,1 mg l^{-1}

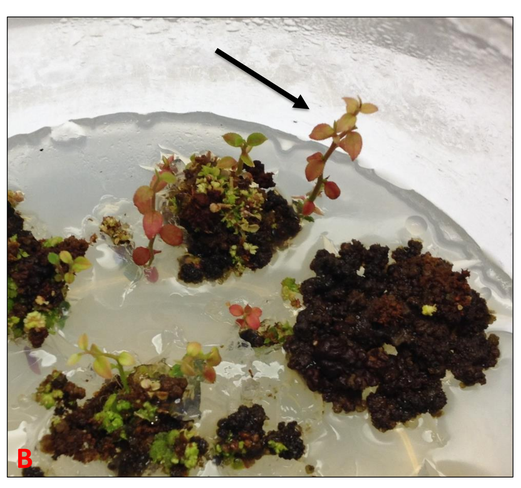
WPM2: + TDZ 0,2 mg l^{-1}

WPM3: + TDZ 0,5 mg l^{-1};

WPM4: + Zeatine 2 mg l^{-1}.







Results(2)

A= leaves direct organogenesis obtained mostly with zeatin (2 mg l^{-1}). B= meristematic bulk indirect organogenesis (via callus formation) was induced with TDZ 0,2 or 0,5 mg l^{-1} (data not shown). In the right photos are shoot regenerated from callus (arrow) ready to be isolated and transplanted in a media without growth regulators.

Conclusions: Zeatin remain the best PGR for inducing blueberry proliferation. TDZ has the negative effect to induce too high callus proliferation. The same is for inducing leaf tissue regeneration. For the first time the meristematic bulk regeneration method was developed in blueberry and in this case TDZ resulted more efficient. Both regeneration approaches can be used for the development of new protocols for blueberry genetic transformation protocols.

References:

Reed B.M., Abdelnour-Esquivel A. (1991). The use of zeatin to initiate in vitro cultures of Vaccinium species and cultivars, Hortic. Sci. 26(10):1320-1322.

Finn C.E., Luby J.J., Rosen C.J., Ascher P.D. (1991) Evaluation in vitro of blueberry germplasm for higher pH tolerance. J. Amer. Soc. Hort. Sci. 116(2):312-316.

Tirone S., Forni C., Lucioli S., Meneghini M., Nota P., De Salvador F.R., Giorgioni M., Catenaro E., Frattarelli A., Ceccarelli D., Caboni E. (2011). Colture in vitro e produzione di composti fenolici in Vaccinium corymbosum L. Acta Italus Hortus 6:211-214.

Song G.Q., Sink K.C. (2006) *Agrobacterium* mediated transformation of highbush blueberry (*Vaccinium corymbosum* L.) cultivars. In: Wang K. (eds) Agrobacterium protocols-methods in molecular biology. Humana, Totowa.

Landi L. and Mezzetti B. (2006). TDZ, auxin and genotype effects on leaf organogenesis in *Fragaria*. Plant Cell Rep. 25(4):281-288.

Mezzetti B., Pandolfini T., Navacchi O., Landi L., (2002). Genetic transformation of *Vitis vinifera* via organogenesis. BMC Biotechnologies 10.1186/1472-6750-2-18.

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