

Influence of plant growth regulators, carbon sources and iron on the cyclic secondary somatic embryogenesis and plant regeneration of transgenic cherry rootstock 'Colt' (*Prunus avium* × *P. pseudocerasus*)

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Abstract

The frequency of long-term secondary somatic embryogenesis and shoot meristem development from embryogenic masses of the cherry rootstock 'Colt' (*Prunus avium* × *P. pseudocerasus*), differentiated from transgenic roots containing the T-DNA of *Agrobacterium rhizogenes*, has opened the way for genetic improvement by biotechnological techniques. Whole plants were produced by stimulating shoot meristem development from somatic embryos. The combination of 4 mg l⁻¹ of kinetin and 2% of maltose under illumination stimulated shoot development and, subsequently, whole plants have been recovered by applying 1.5 mg l⁻¹ kinetin to the rooting medium. Although numerous treatments have been tested involving both embryogenic masses and whole embryos, normal embryo germination was observed sporadically. Cold treatment was effective in stimulating secondary somatic embryogenesis with embryo development to the cotyledonary stage, but did not promote their germination. Similarly, a higher concentration (44–55 mg l⁻¹) of chelated iron than that commonly used in tissue culture media (36.7 mg l⁻¹) produced, after 3 weeks in culture, almost a 50% increase in the number of embryos at the cotyledonary stage per embryogenic mass. Among the cytokinins tested, 1 mg l⁻¹ of 6-benzylaminopurine and 0.1 mg l⁻¹ of thidiazuron were effective in inducing secondary somatic embryogenesis; however, each of them expressed highest efficiency with specific medium and environmental conditions. Furthermore, application of 1 mg l⁻¹ thidiazuron reverted morphogenic callus to non-morphogenic callus, particularly in medium containing 2% sucrose. Finally, hormone free medium with 2% maltose enhanced maturation of the embryos to the normal cotyledonary stage. This paper has improved knowledge of embryo culture and plant production in this important genotype, opening the way for genetic improvement by biotechnological techniques, mainly with the aim of modifying the growth pattern of the canopy of sweet cherry grafted on it.

Abbreviations: BAP – 6-benzylaminopurine; BM – basal medium; 2,4D – 2,4-dichlorophenoxyacetic acid; GA₃ – gibberellic acid; KIN – kinetin; MS – Murashige and Skoog; NAA – naphthalenacetic acid; PGR – plant growth regulators; SSE – secondary somatic embryogenesis; TDZ – thidiazuron

Introduction

Experimental protocols for *in vitro* somatic embryogenesis are not invariant across different plant species, indeed both genotype and tissue culture conditions influence ability to undergo

somatic embryogenesis within a genera (Ammirato, 1983). The choice of medium components can affect development of specific embryogenic stages and their conversion into plants; however, auxins and cytokinins are always required for starting the embryogenic process. In addition to the plant