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Short Communication

Role of salicyclic acid on conifer somatic embryogenesis

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This review paper highlights about the role of salicyclic acid on somatic embryogenesis and also recent updates on cloning mature trees of conifers using salicyclic acid were discussed. Salicyclic acid (SA) is a mobile molecule, which is capable of acting as a cell signal that senses, amplifies, and transmit information from a cell and might help in programming towards embryogenesis during cloning. Very recently incorporation of 1.0 mg^{-1} SA in the DCR induction medium was found to be optimum for all the genotypes of *P. roxburghii* in terms of increasing the percentage of somatic embryogenesis compared against control. Therefore, salicyclic acid can be used as growth regulator in conifer somatic embryogenesis and its use might help to solve the low initiation frequencies of many other recalcitrant pines. However, the mechanism of salicylate-induced differentiation in plants is not known, although salicylate is a signal molecule implicated in eliciting many physiological functions in plants.

Key words: Cloning, conifers, mature trees, forestry, India, signaling

Abbreviations: ASA- acetylsalicyclic acid; DCR- Durzan and Gupta medium; H_2O_2- Hydrogen peroxide; PGR- Plant growth regulators; SA-salicyclic acid; TDZ- thidiazuron; tTCLs-transverse thin cell layers;

Overview

The plant growth regulator, salicyclic acid (SA), when applied to plants, affects diverse physiological processes (Malabadi *et al*. 2008a, 2008b; Cleland and Ajami, 1974; Cleland *et al*. 1982; Conrath *et al*. 1995; Dean and Delaney, 2008; Durner and Klessig, 1995; Gutierrez-Coronado *et al*. 1998; Hao *et al*. 2006; Hutchinson and Saxena, 1996; Komaraiah *et al*. 2004; Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al*. 2001). In African violet, the application of SA at low concentrations affects plant size, and the number of leaves and flowers. Moreover, application of SA to the growth medium induced flowering in several species of Lemnaceae. SA has also been reported to increase the activity of superoxide dismutase, and inhibit activities of ascorbate peroxidase, and catalase, thus leading to endogenous

H_2O_2 accumulation (Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al.* 2001). Thus, SA inhibits the decomposition of H_2O_2 produced in plants. SA is an important signaling molecule involved in plant defense responses to pathogens and abiotic stress, as well as in plant growth and development. There are several reports describing exogenous SA and acetylsalicyclic acid (ASA) enhancing somatic embryogenesis in plants *viz.* carrot (*Daucus carota*), pearl millet (*Pennisetum americanum*), geranium (*Pelargonium* × *hortorum* Bailey), *Astragalus adsurgens* Pall, *Plumbago rosea* L., naked oat (*Avena nuda*), and *Pinus roxburghii*. On the other hand salicyclic acid also promotes induction of flowering in duckweeds, increased cell division, and growth during somatic embryogenesis in tissue culture of *Coffea arabica*, stomatal closure in *Phaseolus vulgaris*, increase in the accumulation of nitrates in roots of *Pinus patula*, inhibition of the biosynthesis of ethylene in cell suspension cultures of pears and seed germination in species, and root growth stimulation of soybean (Malabadi *et al*. 2008a, 2008b; Cleland and Ajami, 1974; Cleland *et al*. 1982; Conrath *et al.* 1995; Dean and Delaney, 2008; Durner and Klessig, 1995; Gutierrez-Coronado *et al.* 1998; Hao *et al*. 2006; Hutchinson and Saxena, 1996; Komaraiah *et al*. 2004; Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al.* 2001; Martin-Max *et al*. 2005; San Miguel *et al*. 2002; Raskin, 1992; Quiroz *et al*. 2001; Ping *et al*. 2001).

Role of salicyclic acid on somatic embryogenesis

SA is an important signaling molecule not only involved in defense responses but also in somatic embryogenesis of many plant species. SA is a mobile molecule, which is capable of acting as a cell signal that senses, amplifies, and transmit information from a cell and might help in programming towards embryogenesis during cloning. Secondly, SA is involved (together with nitrogen oxide, hydrogen peroxide, and other metabolites) in the function of several signal systems, unifying them into an intricate network of regulatory interactions. Perhaps embryo differentiation may sharing some of the intermediates in the salicylate signal pathway(Malabadi *et al*. 2008a, 2008b; Cleland and Ajami, 1974; Cleland *et al*. 1982; Conrath *et al.* 1995; Dean and Delaney, 2008; Durner and Klessig, 1995; Gutierrez-Coronado *et al.* 1998; Hao *et al*. 2006; Hutchinson and Saxena, 1996; Komaraiah *et al*. 2004; Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al.* 2001; Martin-Max *et al*. 2005; San Miguel *et al*. 2002; Raskin, 1992; Quiroz *et al*. 2001; Ping *et al*. 2001).

In a recent study, it was observed that SA acts as a signaling molecule during cloning mature trees of *P. roxburghii*. Therefore, SA can be used as growth regulator in conifer somatic embryogenesis and its use might help to solve the low initiation frequencies of many other recalcitrant pines. Embryogenic cultures have been generated for most of the conifers. For the most part, however, even the best of these systems lack commercial viability for two reasons: first, a low frequency of regeneration for many of the most desirable clones; and secondly, unproven genotypes, as starting material for the cultures is derived from seeds or seedlings. Therefore, the current approach of cloning mature trees of conifers using SA has many practical applications particularly in clonal forestry schemes. Use of SA might be helpful in solving many problems of conifer somatic embryogenesis. The pre-treatment of tTCLs from any of the 10 genotypes of *P. roxburghii* with 0.1, 0.2 and 0.4 mg^{-1} SA could not effectively increasing the percentage of somatic embryogenesis when compared to the control (Malabadi *et al*. 2008a, 2008b; Malabadi and Teixeira da Silva, **2011**). Pretreatment of explants with higher concentrations (2.0-5.0 mg^{-1}) of SA might have had a toxic effect and resulted in the browning of explants without callus

formation in all 10 genotypes. Incorporation of 1.0 mg^{-1} SA in the induction medium was optimum for all 10 genotypes by increasing the percentage of somatic embryogenesis compared to the control. The addition of 1.0 mg^{-1} SA to the induction medium was very beneficial since in the control this genotype failed to induce somatic embryogenesis. This clearly indicates the positive role of SA as a signaling molecule during cloning of mature *P. roxburghii* trees. In this study SA alone (i.e. without PGRs) did not induce somatic embryogenesis and resulted in the browning of explants and callus. Therefore, SA when combined with 22.6 μM 2, 4-D, 26.8 μM NAA, 8.9 μM BAP in the induction medium, improved the percentage of somatic embryogenesis. Hence, the combination of SA with other PGRs such as 2,4-D/NAA/BA might be beneficial in inducing somatic embryogenesis in Chir pine. In geranium (*Pelargonium* x *hortorum* Bailey), thidiazuron (TDZ) effectively induced somatic embryogenesis in cultured hypocotyls explants during only a 3-day period of induction. The presence of acetylsalicyclic acid (ASA) during this period caused a two-fold increase in the number of somatic embryos an enhanced synchronization of embryo development compared to the TDZ treatment alone. However, in the same study, SA was ineffective in modulating similar embryogenic responses as ASA in geranium. Enhanced somatic embryogenesis and plant regeneration have been obtained using young leaf bases of naked oat (*Avena nuda*) as explants by including 0.5 mM SA and carrot embryogenic callus extracts in MS media. An improvement was achieved in somatic embryogenesis and plant regeneration on the corresponding media supplemented with 0.5 mM SA and carrot embryogenic callus extracts as compared to control. Somatic embryogenesis was induced from suspension cultures derived from leaf callus of an important medicinal plant, *Plumbago rosea* L. in which 8.32 μM ASA alone induced embryogenesis, but IAA, NAA or IBA alone failed to elicit a similar response. Optimal embryogenic response per culture (216 embryos per culture) was observed in MS medium containing a combination of ASA (8.32 μM) and IAA (5.06 μM), i.e. a similar synergistic response as observed in our study between SA/ASA and other PGR(s) in the medium. It was also observed that by increasing the concentration of ASA alone (without auxin) in the medium (up to 11.09 μM) the number of somatic embryos formed per culture increased. The interactive effect of ASA and IAA appears to be essential for enhanced production of embryos per culture since no embryogenesis was noticed when IAA alone was added in *Plumbago rosea* L. SA is endogenously produced in many plants in normal as well as during stress conditions. However, the mechanism of salicylate-induced differentiation in plants is not known, although salicylate is a signal molecule implicated in eliciting many physiological functions in plants. It was also reported that the inclusion of SA to differentiation medium below 200 μmol/L significantly enhanced somatic embryogenesis in *Astragalus adsurgens* Pall., callus cultures, the highest frequency of somatic embryogenesis occurring at 150 μmol/L SA. They also reported that enhanced somatic embryogenesis by SA was accompanied by an increase in the endogenous hydrogen peroxide (H_2O_2) level compared to controls (Malabadi *et al*. 2008a, 2008b; Cleland and Ajami, 1974; Cleland *et al*. 1982; Conrath *et al*. 1995; Dean and Delaney, 2008; Durner and Klessig, 1995; Gutierrez-Coronado *et al*. 1998; Hao *et al*. 2006; Hutchinson and Saxena, 1996; Komaraiah *et al*. 2004; Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al*. 2001; Martin-Max *et al*. 2005; San Miguel *et al*. 2002; Raskin, 1992; Quiroz *et al*. 2001; Ping *et al*. 2001).

This increased endogenous H_2O_2 level was related to the inhibition of ascorbate peroxidase and catalase activities. Although

the promoting effect of exogenous H_2O_2 was significantly lower than that of exogenous SA on the development of somatic embryos, the pre-treatment of callus cultures of *A. adsurgens* with dimethylurea (a trap for H_2O_2) significantly inhibited somatic embryogenesis, even if callus was cultured on the differentiation medium supplemented with 150 µmol/L SA, suggesting that endogenous H_2O_2 was required for SA-enhanced somatic embryogenesis in *A. adsurgens* (Malabadi *et al.* 2008a, 2008b; Cleland and Ajami, 1974; Cleland *et al*. 1982; Conrath *et al.* 1995; Dean and Delaney, 2008; Durner and Klessig, 1995; Gutierrez-Coronado *et al.* 1998; Hao *et al*. 2006; Hutchinson and Saxena, 1996; Komaraiah *et al*. 2004; Larque-Saavedra, 1978, 1979; Leslie and Ramani, 1988; Luo *et al.* 2001; Martin-Max *et al*. 2005; San Miguel *et al*. 2002; Raskin, 1992; Quiroz *et al*. 2001; Ping *et al*. 2001).

Therefore, one possible link between oxidative stress and plant regeneration in tissue culture could be H_2O_2. SA is endogenously produced in many plants in normal as well as during stress conditions. SA also inhibited ethylene biosynthesis in cell suspension cultures of carrot. It is well known that ethylene inhibits differentiation in plants. Therefore, SA may be promoting embryo development by inhibiting ethylene biosynthesis. Another hypothesis is that SA has been reported to increase the activity of superoxide dismutase, and inhibits the activities of ascorbate peroxidase and catalases, thus leading to endogenous H_2O_2 accumulation in *Arabidopsis thaliana*.

References

Cleland CF, Ajami A (1974) Identification of the flower-inducing factor isolated from aphid honeydew as being salicyclic acid. *Plant Physiology* **54**, 909-906

Cleland CF, Tanaka O, Feldman IJ (1982) Influence of plant growth substances and salicyclic acid on flowering and growth in the Lemnaceae (duckweeds). *Aquatic Botany* **13**, 3-20

Conrath U, Chen Z, Ricigliano JR, Klessig DF (1995) Two inducers of plant defense responses, 2, 6-dichloroisonicotinic acid and salicyclic acid inhibit catalase activities in tobacco. *Proceedings of National Academy of Sciences USA* **92**, 7143-7147

Dean JV, Delaney SP (2008) Metabolism of salicyclic acid in wild-type, ugt74f1 and ugt74f2 glucosyltransferase mutants of *Arabidopsis thaliana*. *Physiologia Plantarum* **132**, 417-425

Durner J, Klessig DF (1995) Inhibition of ascorbate peroxidase by salicyclic acid and 2, 6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proceedings of National Academy of Sciences USA* **92**, 11312-11316

Gutierrez-Coronado M, Trejo CL, Larque-Saavedra A (1998) Effects of salicyclic acid on the growth of roots and shoots in soybean. *Plant Physiology Biochemistry* **36** (8), 563-565

Hao L, Zhou L, Xu X, Cao J, Xi T (2006) The role of salicyclic acid and carrot somatic embryogenic callus extracts in somatic embryogenesis of naked oat (*Avena nuda*). *Plant Cell Tissue and Organ Culture* **85**, 109-113

Hutchinson MJ, Saxena PK (1996) Acetylsalicyclic acid enhances and synchronizes thidiazuron induced somatic embryogenesis in geranium (*Pelargonium* x *hortorum* Baily) tissue cultures. *Plant Cell Reports* **15**, 512-515

Komaraiah P, Jogeswar C, Ramakrishna S, Kavi Kishor P (2004) Acetylsalicyclic acid and ammonium induced somatic embryogenesis and enhanced plumbagin production in suspension cultures of *Plumbago rosea* L. *In Vitro Cellular and Developmental Biology-Plant.* **40** (2), 230-234.

Larque-Saavedra A (1978) The antitranspirant effect of acetylsalicyclic acid on *Phaseolus*

vulgaris L. *Physiologia Plantaram* **43**, 126-128

Larque-Saavedra A (1979) Stomatal closure in response to acetylsalicyclic acid treatment. *Z. Pflanzenphysiol* **93** (4), 371-375

Leslie CA, Romani RJ (1988) Inhibition of ethylene biosynthesis by salicyclic acid. *Plant Physiology* **88**, 833-837

Luo JP, Jiang ST, Pan LJ (2001) Enhanced somatic embryogenesis by salicyclic acid of *Astragalus adsurgens* Pall: relationship with H_2O_2 production and H_2O_2-metabolizing enzyme activities. *Plant Science* **161**, 125-132

Malabadi RB, Teixeira da Silva J, Nataraja K (2008a) A new approach involving salicyclic acid and thin cell layers for cloning mature trees of *Pinus roxburghii* (Chir Pine). *The Americas Journal of Plant Science and Biotechnology.* **2**(2):56-59.

Malabadi RB, Teixeira da Silva J, Nataraja K **(**2008b) Salicylic acid induces somatic embryogenesis from mature trees of *Pinus roxburghii* (Chir pine) using TCL Technology. *Tree and Forestry Science and Biotechnology*. **2**(1): 34-39.

Malabadi RB, Teixeira da Silva J (**2011**) Thin cell layers: Application to forestry biotechnology. *Tree and Forestry Science and Biotechnology***. 5**(1): 14-18.

Martin-Mex R, Villanueva-Couoh E, Herrera-Campos T, Larque-Saavedra A (2005) Positive effect of salicyclates on the flowering of African violet. *Scientia Horticulturae* **103**, 499-502

Ping LJ, Tong JS, Jun PL (2001) Enhanced somatic embryogenesis by salicyclic acid of *Astragalus adsurgens* Pall: relationship with hydrogen peroxide production and hydrogen peroxide-metabolizing enzyme activities. *Plant Science* **161**, 125-132

Raskin I (1992) Role of salicyclic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 439-463

San Miguel R, Gutierrez M, Larque-Saavedra A (2002) Low concentration of salicyclic acid increase nitrate accumulation in roots of *P. patula*. *Phyton* 79-82

Quiroz FM, Mendez ZM, Larque-Saavedra A, Loyola-Vargas VM (2001) Picomolar concentration of salicyclic induce cellular growth and enhance somatic embryo-genesis in *Coffea arabica* tissue culture. *Plant Cell Reports* **20**, 679-684