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Moderation of morphogenetic and oxidative stress responses in flax in vitro cultures by hydroxynonenal and desferrioxamine

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Summary

Hypocotyl segments of 7-day old seedlings of flax (*Linum usitatissimum* L.) cultivars Atalante, Flanders, Jitka, Szegedi 30 and Super were screened for organogenesis (shoot and root induction) and embryo-like structure production. A non-destructive assay for hydroxyl radicals (•OH), utilising DMSO as a radical trap, was used to determine •OH formation during tissue culture and morphogenesis. Desferrioxamine, an inhibitor of Fenton reaction, and 4-hydroxy-2-nonenal, a cytotoxic lipid peroxidation product, were exogenously applied to flax cultures to determine the effect of antioxidative and prooxidative status on morphogenetic responses induced through the exogenous application of plant growth regulators.

Flax genotypes varied in their response to treatments after exposure to different plant hormones. Hydroxyl radical (•OH) formation correlated with morphogenetic responses and this was affected by plant hormones. Desferrioxamine and 4-hydroxy-2-nonenal also moderated morphogenetic responses and influenced hydroxyl radical formation during in vitro propagation.

Abbreviations: BAP, benzyl aminopurine; DMSO, dimethyl sulfoxide; ELS, embryo-like structure; HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; NAA, naphthaleneacetic acid

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Introduction

Flax (*Linum usitatissimum* L.) is an important crop for the production of oil and fibre and in vitro manipulations are an essential component of genetic improvement and flax breeding.

Somatic embryos of flax were first time derived from immature zygotic embryos (Pretova and Williams, 1986) and subsequently from the hypocotyl segments of in vitro – grown flax seedlings (daCunha and Ferreira, 1996; Dedicova et al., 2000; Tejavathi et al., 2000). Although a high regeneration rate was achieved, understanding of those factors that affect and control in vitro morphogenesis in this species is limited and often contradictory. This restricts the implementation of biotechnology programmes aimed at flax improvement. For example, somatic embryos derived from flax hypocotyl segments regenerate at only limited rates, largely due to abnormal development and morphology (Dedicova et al., 2000).

Oxidative processes may affect the morphogenic responses of cells grown in vitro (Benson and Roubelakis-Angelakis, 1994) and lipid peroxidation has been associated with early dedifferentiation in *Vitis vinifera* (Benson and Roubelakis-Angelakis, 1992, 1994). Free radicals may be implicated in in vitro plant recalcitrance and ageing (Benson, 2000a, b) and this may be related to abnormal metabolism (Gaspar et al., 2000).

Damage caused by free radicals may thus be a factor in promoting stress and ageing in plant tissue cultures (Benson, 2000a, b; Van der Linde, 1990). Indirect evidence indicates, that •OH and $O_2^{\bullet -}$, both highly toxic oxygen species, are produced by dedifferentiated plant cultures (Benson and Withers, 1987).

Oxidative stress has been proposed to contribute to recalcitrance of plant protoplasts (Kapur et al., 1993; Papadakis et al., 2001). Reactive oxygen species, ROS (superoxide $O_2^{\bullet -}$ radicals, hydrogen peroxide H_2O_2 and hydroxyl radicals •OH) can accumulate in response to biotic and abiotic stress. They can have a detrimental effect on the metabolism, growth and development through their ability to initiate reaction cascades. These result in the production of toxic chemical species such as lipid peroxides and their aldehydic breakdown products leading to cell dysfunction and death (Alscher et al., 1997). In parallel, however ROS may also have a positive role in plant growth and development; for example, $O_2^{\bullet -}$ and H_2O_2 may serve as signal molecules, stimulating defense responses (Jabs et al., 1997; Lamb and Dixon, 1997).

The balance between essential and damaging oxidative reactions is influenced by the physiological and developmental status of tissues and exogenous factors such as stress, disease or wounding and the application of plant growth regulators (Benson, 2000a, b). The introduction and proliferation of plants or their parts in vitro may alter oxidative metabolism and predispose tissues to the damaging effects of reactive oxygen species. A dual role for H_2O_2 has been shown in plant protoplast division and regeneration. Thus in the case of *V. vinifera*, H_2O_2 reduces regeneration potential, but contrastingly H_2O_2 is required for protoplast division (de Marco and Roubelakis-Angelakis, 1996a, b). Data from comparative work using tobacco and grapevine protoplasts have shown significant differences in their oxidative and antioxidative machinery (Papadakis et al., 2001).

Studies of lipid peroxidation in plant tissue cultures demonstrated that toxic aldehydes are produced during culture initiation and throughout routine subculturing (Benson and Roubelakis-Angelakis, 1994; Robertson et al., 1995).

It has been confirmed, that HNE is produced de novo by plant cells and that the exogenous application of the aldehyde can inhibit in vitro growth and development (Bremner et al., 1997; Deighton et al., 1997). Differences between embryogenic and non – embryogenic callus regarding the endogenous level of MDA and HNA showed that embryogenic callus contained higher level of both aldehydes, and this could reflect the enhanced morphogenetic competence of these cultures (Adams et al., 1999). Clearly, the aforementioned studies indicated that free radical – mediated reactions could moderate in vitro ageing, development and morphogenesis.

The overall objective of this study is therefore: to explore the possibility that free radical mediated oxidative stress may have a role in moderating in vitro and morphogenetic development in flax.

Two different approaches have been selected, which target studies of both primary and secondary phases of the oxidative stress cascade.

The first approach concerns the exogenous application of desferrioxamine, an inhibitor of Fenton and Haber–Weiss reaction and this moderates the initiation of a free radical reaction cascade (Halliwell and Gutteridge, 2003).

Desferrioxamine is an iron chelating agent, produced by *Streptomyces pilosis*, which is a powerful chelator of Fe^{III}. It is able to inhibit iron salt-dependent lipid peroxidation and the generation of highly reactive oxidising species from