

Influence Of Acetyl Salicylic Acid In Combination With Fish Protein Hydrolysates On Hyperhydricity Reduction And Phenolic Synthesis In Oregano (*Origanum Vulgare*) Tissue Cultures

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Abstract

Tissue culture-generated shoot-based clonal lines of oregano are being used to investigate the role of proline-linked pentose phosphate pathway in stimulating the phenolic antioxidant, rosmarinic acid (RA) and lignification, which was reported to be linked to guaiacol peroxidase (GPX) activity. One of the problems in oregano tissue culture is hyperhydricity and modifications of media are being used to control this physiological malformation. This study reports the reduction of hyperhydricity, stimulation of RA biosynthesis and lignification in oregano clonal line O-1 and O-5 in response to acetyl salicylic acid (ASA), fish protein hydrolysate (FPH, standardized mackerel hydrolysates) and combination of FPH and ASA (FPH/ASA).

All treatments reduced hyperhydricity of both clonal lines compared to control. Following exogenous treatment with ASA and FPH/ASA, enhanced total phenolic content, RA content and concurrently higher levels of GPX activity were observed compared to control and FPH treatments. The concentration of total phenolics and RA as well as GPX activity in O-5 clonal line was higher than in O-1 clonal line either on day 15 or day 30. Antioxidant activity of the phenolic extracts of all cultures was high on day 30 compared to day 15 and FPH/ASA treatment had the highest activity on both days. The concentration of chlorophyll in O-1 was higher than in O-5 and the concentration in both lines were similar in response to respective treatments.

The stimulation of RA synthesis in response to ASA and FPH/ASA provided strong clues that ASA can be used as an abiotic elicitor for RA stimulation and mobilization of proline and/or glutamic acid in FPH and this may be linked to stimulation of the pentose phosphate pathway, driving key precursor metabolites towards shikimate and phenylpropanoid pathways. RA-stimulating compounds also enhanced total phenolics and hardened stem tissues, which correlated with higher GPX activity, indicating possible lignification due to polymerization of phenolic metabolites. This research also provides strategies to prevent hyperhydricity in tissue culture by ASA and combination of FPH/ASA and these were linked to lignification, high levels of total phenolics and RA. This improvement is important for efficiency and quality of in vitro

plant tissue propagation and outdoor transplanting of elite phenolic antioxidant-producing oregano cultures. This research also provides insight into regulation of rosmarinic acid, a phenolic antioxidant relevant for food preservative applications.

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