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Paclobutrazol and GA₃ synergistically promote somatic embryogenesis from root apices of spinach

PP1-10

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Gibberellic acid (GA₃) is indispensable for somatic embryo (SE) induction from root apices of spinach. To elucidate its role in SE induction, paclobutrazol (PAC), an inhibitor of gibberellin biosynthesis, was used. Root apices (1 cm) isolated from SE-derived, in vitro-cultivated plants of a previously selected line with high embryogenic capacity were used for experimentation. The explants were cultivated on media containing 20 μ M α -naphthaleneacetic acid (NAA) + 0 or 5 μ M GA $_3$ + 0, 1, 2.5, 5 or $10 \mu M$ PAC. NAA alone induced SE regeneration in 89% of the explants, but with only 3.5SEs per explant, while the explants cultivated on media supplemented with NAA+GA₃ or NAA + $GA_3 + 2.5 \mu M$ PAC regenerated at 100% with 17.7 and 34.6 SEs per explant, respectively. However, in the absence of GA₃, higher levels of PAC were needed: 5-10 µM PAC + NAA for 100%-response and 15-22.2 SEs per explant. To get insight into the NAA-GA₃-PAC interaction, expression of genes encoding the key enzymes that catalyze the final step of bioactive GA biosynthesis (GA20-oxidase and GA3-oxidase) and degradation (GA2-oxidase) was analyzed in the explants during 28 days of SE-induction period, using quantitative real-time PCR. In the explants with high embryogenic capacity, the expression levels of SoGA20 ox and SoGA3 ox were significantly lower, and SoGA2ox1 and SoGA2-ox3 significantly higher than in those with low embryogenic capacity, cultivated on medium with NAA alone, during the whole SE induction period, indicating that NAA-GA₃-PAC interaction enabled the acquisition of embryogenic capacity by impacting GA metabolism.

Keywords: paclobutrazol, somatic embryogenesis, gibberellins, gene expression, Spinacia oleracea

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