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Elucidation of the mechanism underlying somatic embryo induction in spinach

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Spinach is a dioecious plant species with complex sex determination, which limits the success of its classical breeding. Hence, a biotechnological approach has been recognized as a promising alternative. Elucidation of the mechanism underlying somatic embryo (SE) induction and improvement of its efficiency have been the main goals of many years of research in this plant species recalcitrant to in vitro regeneration. Results have evidenced that the interplay among auxin, gibberellins (GA) and light was crucial for the induction of somatic embryogenesis from the root apices. None of these factors can be substituted for each other and only the lines with inherited high embryogenic capacity could respond in the absence of any of them, but with a very limited efficiency. The highest embryogenic response was obtained from the explants cultivated on medium supplemented with 20 μM α naphthaleneacetic acid (NAA) + 5 μM gibberellic acid (GA₃) under blue light. Contrary to expectations, paclobutrazol, an inhibitor of bioactive gibberellin biosynthesis, acted synergistically with NAA and GA₃ and further promoted somatic embryogenesis. In embryogenic explants, the expression levels of genes encoding the key enzymes involved in GA biosynthesis decreased, while those of genes involved in GA inactivation increased compared to non-embryogenic ones. Analysis of GA content using ultra-high performance liquid chromatography revealed enhanced GA metabolism in embryogenic explants during the first week of SE induction. During this period, the levels of endogenous bioactive GAs (GA1, GA3, GA4 and GA7) dramatically increased, confirming their important role in the acquisition of embryogenic potential.

Keywords: spinach, somatic embryogenesis, gibberellins, gene expression, paclobutrazol

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