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Development of chestnut (*Castanea sativa Mill.*) micro-propagation through zygotic embryogenesis

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To establish an effective protocol for plant regeneration through zygotic embryogenesis effects of explant, culture media and plant growth regulators on chestnut (*Castanea sativa Mill.*) regeneration were investigated. Explant (zygotic embryo), two different media Murashige and Skoog medium (MS) and Woody Plant Medium (WPM), different plant growth regulators (6-benzylaminopurine (BAP), Indole-3-Butyric Acid (IBA) with different concentration (0.1, 0.2 and 3.0 mgL^{-1}) and phenol inhibitors (activated charcoal, citric acid, Polyvinylpyrrolidone (PVP)) for shoot and root induction were chosen. The culture of chestnut showed the better initiation and multiplication rates in WPM medium. 0.1 mgL^{-1} BAP in combination with activated charcoal was the best growth regulator for shoot elongation. WPM supplemented with 0.1 mgL-1 BAP and citric acid was the best shoot proliferation. Rooting of *in vitro* plantlets was achieved on WPM medium supplemented with 3.0 mgL^{-1} IBA showing 60% intensity and was the most efficient in terms of secondary root production. For acclimatization rooted plants were transferred to plastic pots filled with mixture of sphagnum peat:perlite at the ratio 2:1. This protocol provides a basis for future studies on protection of rare and endangered plant species using biotechnological approaches, thus preserving diversity of the forest ecosystems.

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