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PLANT TISSUE CULTURE

Induction of multiple shoots in *Oryza sativa*: roles of thidiazuron, 6-benzylaminopurine, decapitation, flooding, and Ethrel® treatments

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Abstract

The impact of different concentrations of thidiazuron (TDZ) and 6-benzylaminopurine (BAP) and the role of decapitation, flooding, and Ethrel® treatments were evaluated on direct *in vitro* caryopsis culture of rice. After 28 d of culture, multiple-shoot formation was observed from 50 to 90% of TDZ-treated seedlings. A similar but lower frequency response was observed using BAP-supplemented media. Furthermore, multiple shoots appeared to arise from the mesocotyl region of the seedlings. In subsequent experiments, isolation and transfer of mesocotyl segments to TDZ-supplemented media resulted in increased number of multiple shoots (10 to 12) as compared to intact seedlings (5 to 6). The formation of multiple shoots per seedling (13 to 15) and percent responding cultures with multiple shoots (70%) increased when decapitated rice seedlings were used instead of intact seedlings or mesocotyl segments. This demonstrated a possible role of physical stress in multiple-shoot formation along with other factors. Multiple shoots per seedling and percent responding cultures with multiple shoots also increased when intact seedlings were flooded with TDZ-supplemented media; however, flooding with distilled water and N_6 had a very minor effect. Furthermore, the addition of Ethrel® to the culture media, which is metabolized within plant tissue to release ethylene, also resulted in multiple-shoot formation from seedlings. Based on these multiple lines of evidence, this study proposes that TDZ is acting to enhance multiple-shoot formation in this rice system through activation of stress-related gene(s) and signaling molecules.

Keywords Caryopsis culture · Ethylene · Mesocotyl · Multiple-shoot · Physical stress · Rice seedlings

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops, and is consumed by more than 3 billion people every day, including over 70% of the world's poor. Rice is the world's second most extensively farmed cereal, after wheat (Gutaker *et al.* 2020). In India, rice is the main food crop consumed by all the people. Rice is a semi-aquatic plant that may be cultivated in submerged, irrigated, rain-fed lowland, and/or highland habitats in both tropical and temperate climates (Soni *et al.* 2018). The majority of rice cultivars can be placed within two subspecies: *Oryza sativa* ssp. japonica and *Oryza sativa* ssp. indica (Gutaker *et al.* 2020). In addition to its worldwide importance as a food crop, rice is also important as a monocot model system for genetic transformation and gene editing *via* tissue culture techniques.

Cytokinins were initially identified in the 1950s by researchers seeking to explain the growth-promoting effect of autoclaved herring-sperm DNA (Wybouw and De Rybel 2019). It was obvious from the beginning that these substances have a significant impact on plant growth, especially when combined with auxin. Typically, a high auxin-to-cytokinin ratio promotes dedifferentiation and callus formation, while a high cytokinin-to-auxin ratio stimulates shoot regeneration. Cytokinins also play a crucial function in response to biotic and abiotic stresses, as well as mediating responses to a variety of other external factors affecting plants (Cortleven *et al.* 2019).

In vitro regeneration of shoots, supported by cytokinin, has been reported to be a stress-related response in some plants (Verdus *et al.* 1997; Mundhara and Rashid 2001; Gairi and Rashid 2002; Pasternak *et al.* 2002; Ikeda-Iwai

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