



# Stress-induced somatic embryogenesis on seedlings of *Azadirachta indica* A. Juss. by thidiazuron and its inhibition by ethylene modulators

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## Abstract

A low concentration of thidiazuron (TDZ) (1  $\mu$ M) is effective in the induction of somatic embryos (SEs) on different parts of seedlings of *Azadirachta indica* A. Juss (Neem). Somatic embryogenesis is a consequence of the inhibition of root formation and inhibition in the elongation of hypocotyls and epicotyl. This is suggestive that TDZ is effective in the induction of ‘stress-ethylene’. The putative role of ethylene and consequently its functions during induction upon stress on the somatic embryos of neem became clear with the exogenous application of ethylene-action inhibitors like AgNO<sub>3</sub>, or inhibitors of ethylene-biosynthesis such as CoCl<sub>2</sub> or salicylic acid (SA). Of these, the ethylene biosynthesis inhibitors (CoCl<sub>2</sub> and SA) were found to be more effective as inhibitors of regeneration than AgNO<sub>3</sub>. The inhibition of somatic embryogenesis was more significant when KMnO<sub>4</sub> or KOH, the effective absorbers of ethylene, were added to the culture media. This research paper focuses on the stimulation of somatic embryogenesis in neem by TDZ-induced stress ethylene and later its inhibition by using ethylene inhibitors.

**Keywords** *Azadirachta indica* A. Juss · Ethylene · Neem · Seedling · Somatic embryos · Stress · Thidiazuron (TDZ)

## Introduction

*Azadirachta indica* A. Juss. (thereon *A. indica* or neem) belongs to the family Meliaceae and is a native of India and is considered one of the multipurpose trees of the Indian subcontinent (Gupta et al. 2017) and is also found in southern parts of Asia and Africa (Islas et al. 2020). It is being used in various traditional medicinal formulations since ancient times and is one of the first medicinal plants to be mentioned in Siddha medicine (Gupta et al. 2017). It possesses antiseptic, anthelmintic, and insecticidal properties due to the presence of a plethora of chemical constituents such as c-seco tetranortriterpenoid and azadirachtin. Neem twigs are used for cleaning teeth, flowers are used against bile disorders, leaves for ulcers, and barks for psychiatric, paralysis, and nervous disorders. It is also known to contain anti-inflammatory, anti-angiogenic, and other curing abilities (Gupta et al. 2017; Islas et al. 2020). The seed oil is a rich source of azadirachtin, and oil cake acts as a good fertilizer as well as an insect repellent. Azadirachtin yields are, however, variable, being dependent on plant ecotype and environment (Ermel et al. 1987). Therefore, due to its medicinal properties and other functional benefits, mass production of neem is required for more production of

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