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TDZ-induced triple-response and shoot formation on intact seedlings of *Linum*, putative role of ethylene in regeneration

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Abstract

Intact seedlings of *Linum* regenerate to form shoots on hypocotyl on TDZ medium. Prior to regeneration, these seedlings were characterized by 'triple-response'; inhibition of root elongation, shortening and swelling of hypocotyl and tightening of cotyledons towards apex. Triple-response due to TDZ is a new record, it is similar to ethylene-action and points toward the mode of TDZ action which results in production of 'stress-ethylene' and is followed by shoot regeneration. Putative role of ethylene in regeneration, became apparent when inhibitors of ethylene-action ($AgNO_3$, 10 μM) or ethylene-biosynthesis ($CoCl_2$, 10 μM) resulted in reduction of regeneration response. Also inclusion of $KMnO_4$, an effective absorber of ethylene in culture atmosphere, resulted in inhibition of regeneration. A correlation exists between root repression and shoot promotion by TDZ. BAP also resulted in root repression and shoot formation but at higher concentration (10 μM). TDZ-promoted regeneration is a calcium-mediated response. On calcium-free medium, TDZ induced a low frequency shoot-buds, which failed to develop into leafy shoots. However, this frequency increased on a corresponding increase in concentration of calcium. When seedlings were subjected to calcium-stress, by germinating seeds on calcium-free medium, and on transfer to calcium- and TDZ-fortified medium (1 μM) there was an increased frequency of shoot formation not only on hypocotyl but also on cotyledonary-node, which normally remains quiescent. Inhibitors of calmodulin (CPZ or TFP) inhibited shoot formation on TDZ medium but also promoted elongation of roots. Elongation of root by CPZ or TFP is new finding of this investigation. It also supports the suggestion that root repression and shoot promotion are inversely related.

Keywords: Calcium-deprivation; Calmodulin-inhibitors; Chlorpromazine; Cobalt chloride; Cotyledonary-node; Ethylene; Hypocotyl; Potassium permanganate; Shoot-bud; Silver nitrate; Thidiazuron; Trifluoperazine; Triple-response

1. Introduction

The common flax, *Linum usitatissimum* is one of the oldest and dual purpose crop, a source of oil and fibres. Flax seedlings are characterized by an unusual developmental response, on decapitation the hypocotyl regenerates shoot-buds. The regeneration capacity of hypocotyl segments increases with an increasing distance from apex, it is promoted by cytokinin and accentuated by thidiazuron [1,2].

Induction of shoot-buds on decapitated *Linum* seedlings is a stress-related response [3,4]. Transient stress of different types effective in induction of shoot-buds on hypocotyl of seedlings were heat-, salt- and mineral-stress, abscisic acid- and proline-treatment. A synergism is possible between different types of stress. High-frequency of shoot-bud formation occurred when mineral-stress was combined with decapitation [4]. Mineral-deprived seedlings followed by decapitation and flooding resulted in a still higher number of shoot-buds [5].

Regeneration is also possible on intact seedlings of *Linum*. Induction of meristems, the shoot-bud precursors, was possible on intact non-hormone treated plants on a transient withdrawal of calcium [3]. Under in vitro condition a transient withdrawal of calcium for 5 days (2 days of seed germination and 3 days of seedling growth)

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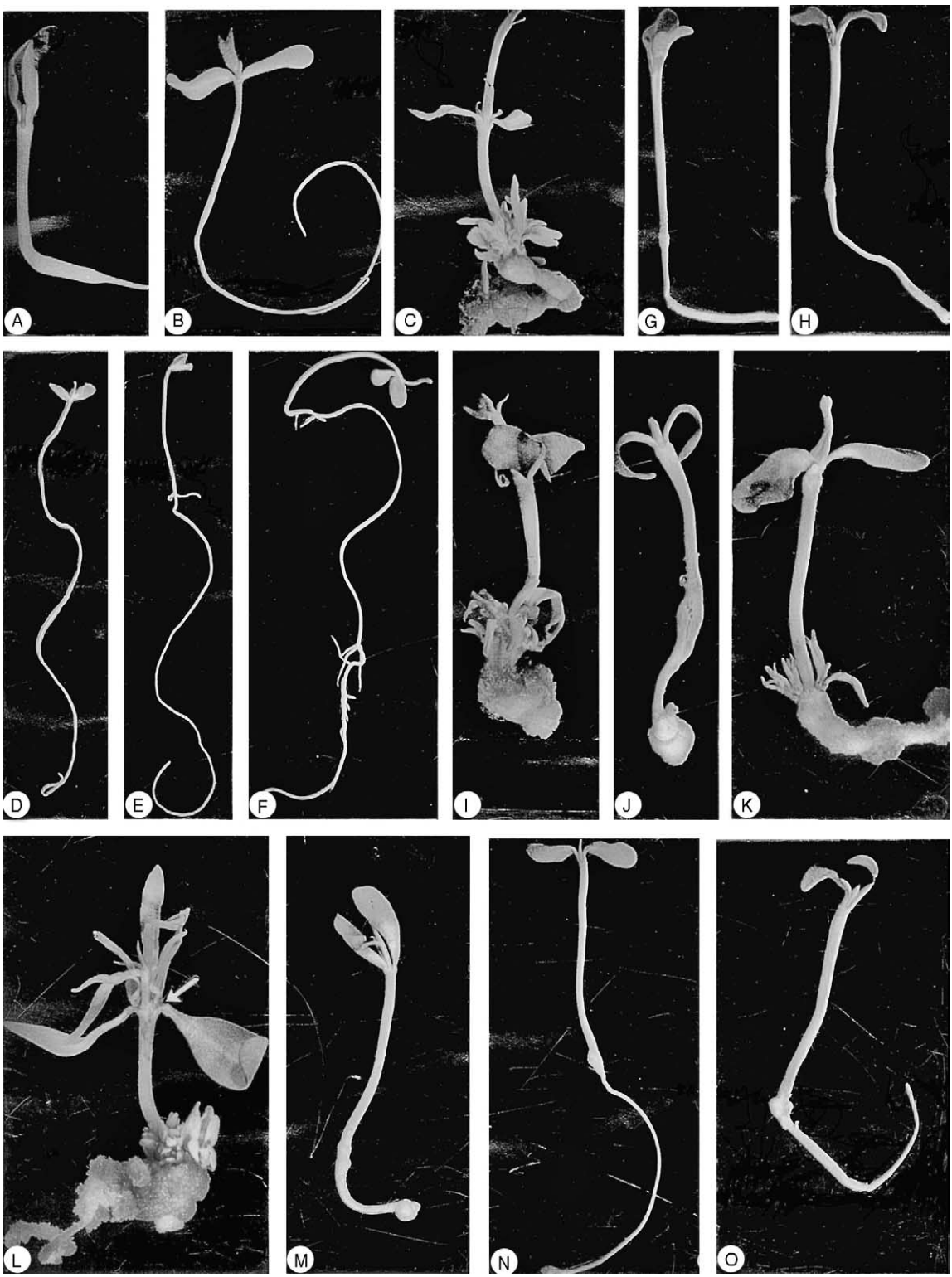

Fig. 1. Induction of shoots on hypocotyl of intact seedlings of *L. usitatissimum* by TDZ or BAP and inhibition by calmodulin and ethylene inhibitors. (A) Four-day-old seedling raised on N_6 + 1 μM TDZ, showing triple-response; (B) the same, raised on hormone-free (N_6) medium, control; (C) 25-day-old seedling raised on N_6 + 1 μM TDZ, showing shoot formation at the swollen basal end of hypocotyl; (D) the same, raised on N_6 medium (control); (E) the same, raised on N_6 + 1 μM chlorpromazine (CPZ), showing elongation of root; (F) the same, raised on N_6 + 1 μM trifluoperazine (TFP), showing elongation and branching of root; (G) the same, raised on N_6 + 1 μM TDZ + 1 μM CPZ, showing elongation of root as compared to control (C); (H) the same, raised on N_6 + 1 μM TDZ + 1 μM TFP, showing elongation of root; (I) the same, raised on N_6 + 10 μM BAP, showing reduction of root length and shoot formation at the swollen end of hypocotyl; (J) the same, raised on calcium-free N_6 medium + 1 μM TDZ, showing one to two initiated shoots; (K) seedling raised on 88 mg/l calcium + 1 μM TDZ, showing development of leafy shoots; (L) calcium-stressed seedling, on transfer to N_6 + 1 μM TDZ, showing development of many