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Enhanced androgenesis in Datura metel by temperature gradient treatment of anthers

When cultured *in vitro*, immature pollen (microspores) undergo embryogenesis and produce haploid plants. Such a haploid induction requires imposition of a stress on microspores. Temperature stress investigated so far has been restricted to a single temperature regime whether high or low. The objective was to study temperature gradient as a stress factor in inducing androgenesis. We report here the pre-treatment of *Datura metel* anthers ranging from 55-10 ^{o}C in quick succession for a short time period of 30 seconds.

Flower buds of *Datura metel* with anther lengths 1.2-1.5 cm (corresponding to mid to late uninucleate stage) were surface sterilized and dissected under aseptic conditions to obtain anthers. Two of the five anthers were cultured directly on the Nitsch (1969) medium supplemented with 1 mg/L kinetin, which served as controls. The remaining three were subjected to a temperature gradient by immersing in hot (45 ^{o}C) and cold (10 ^{o}C) water for 30 seconds each. The procedure was repeated for 50-10 ^{o}C and 55-10 ^{o}C and for comparisons anthers were treated separately at 45 ^{o}C, and at 10 ^{o}C for the same time duration. Both treated and control anthers from a single bud were plated onto the same petri dish and incubated at 23±2 ^{o}C and at 16 h/ 8 h light/dark photoperiod (450 lux). Results were recorded after eight weeks from culture initiation where the total number of embryos produced by each anther were counted under a dissecting microscope and recorded separately.
Out of the three combinations, 45-10 ^{o}C which created a temperature gradient of 35 ^{o}C, produced significantly higher number of embryos than the control ($P<0.05$). Single temperature regimes also produced higher embryogenesis than their respective controls but not as significant as when combined. This confirmed an enhancement effect in embryogenesis when the anthers were treated with the temperature combination to create a gradient. Sudden change of temperatures within quick successions may bring about favorable physiological and biochemical changes in pollen that support androgenesis. Anthers treated with 55 ^{o}C and 50 ^{o}C produced lesser number of embryos than their respective controls suggesting temperature exposure has to be restricted to a physiologically acceptable limit.