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High frequency *in vitro* regeneration from hypocotyl explants of leafy perilla (Perilla *frutescens* L.)

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Objective

Establishment of an efficient regeneration system for leafy perilla to pave the way of further genetic manipulation.

Materials and methods

The seeds of leafy perilla (cv. Manbaeg) were surface sterilized with 70% (v/v) ethanol for 1 minute and washed with sterile distilled water for 3 times. The seeds were then treated with 0.1% (w/v) mercuric chloride for 15 minutes accompanied by moderate shaking by a mechanical rotor followed by washing 3 times as above. The treated seeds were placed on 1/2 MS (Murashize and Skoog) media after blotting with sterile tissue paper and incubated for 7 days at 26±2℃ under cool-white fluorescence lighting conditions. The long hypocotyls from germinated seeds were aseptically excised and cut into basal, middle, apical segments (Fig. 1A) of 0.5 to 1.0cm length and plated separately on MS media containing 0, 2, 4, 6, and 8ppm of BA (6-Benzylaminopurine). The explants were sub-cultured at every 2 weeks interval. A small portion of the hypocotyls attached with regenerated shootlets were excised and placed on MS media for root induction. After 1 to 2 weeks , the plantlets with well- developed roots were placed on sterile distilled water for a one week to acclimatize at 26±2℃ and then transferred onto sterile soil (compost : vermiculite = 1:1) in a glass house.

Results and Discussion

The regeneration capacity of different segments of hypocotyls as explants and their responses to different BA concentrations exhibited wide variations. The apical segments appeared to be the best explant source for accelerated and spontaneous shoot regenerant compared to middle and basal segments when placed on MS media supplemented with 2ppm BA. The optimum condition for satisfactory rhizogenesis of the regenerated leafy perilla shoots was MS media without hormone .

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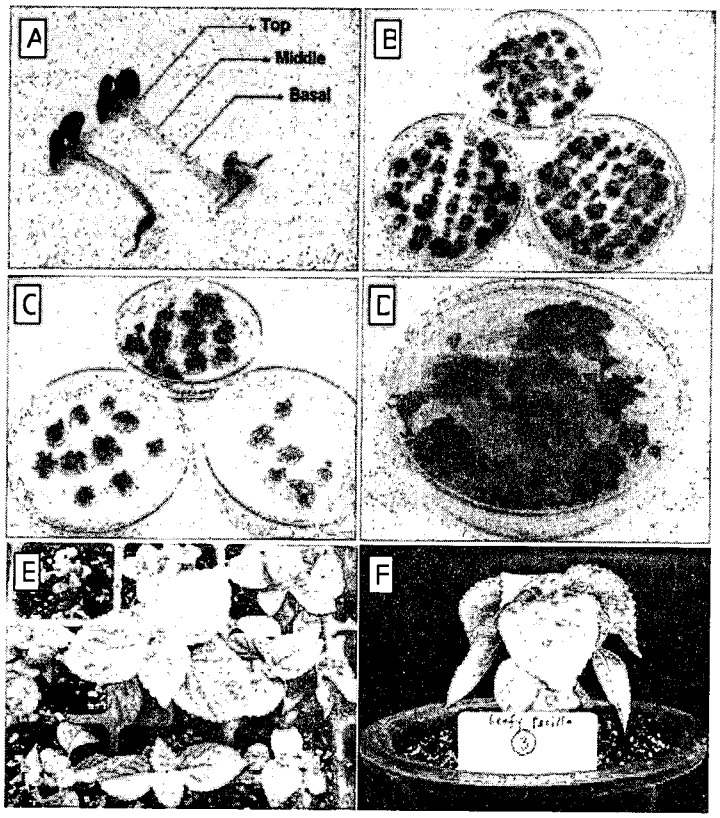

Fig. 1: Regeneration of leafy perilla plants using hypocotyl as explants. (A) Segments of hypocotyl from 6-7 days old seedlings. (B) Explants on growth media; top, bottom-left, and bottom-right plates contain the top, middle, and basal segment of hypocotyls respectively. (C) The same explants of plates in fig. B after removal of calli. (D) Induced shoots on rooting media. (E) Regenerated plants in small pot soils. (F) Mature plant growing in a glass house.

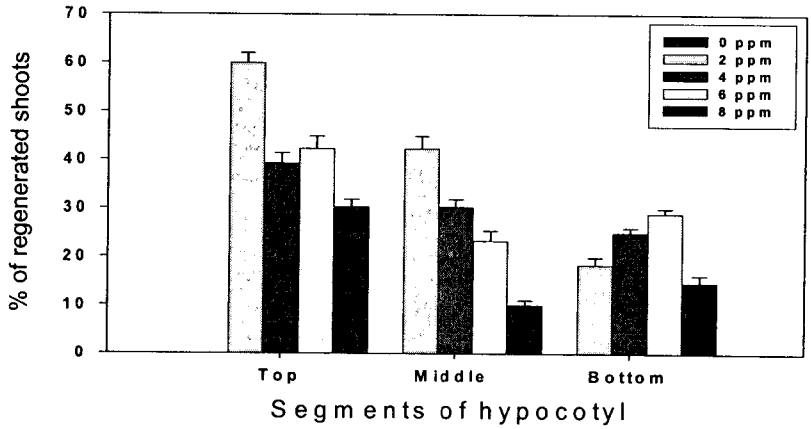

Fig. 2: Effects of hypocotyl segment location and concentrations of BA on regeneration efficiency of leafy perilla. The vertical bars indicate the standard deviations of 40 explants replication measurements.