SUMMARY

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The results of experimental work conducted on *Withania somnifera* (L.) Dunal are summarised in this chapter.

W. somnifera vegetative and fertile plants showed the presence of an alkaloid tropine, which was isolated in their chloroform extracts. Vegetative plants accumulated 0.10% of tropine alkaloid whereas in fertile plants it increased to 0.43%. Out of the samples of plants collected from various localities, Ellora Park area plants exhibited highest tropine (0.55%) in their fertile stage of development. Hence, these plants were identified as superior 'elite' plants. Highest amount of alkaloid accumulated in the roots (0.25%) when compared with other organs *viz.*, stem (0.10%), leaves (0.13%) and fruits (0.08%). The results showed that maximum tropine was synthesized/ accumulated in roots of this plant.

In vitro excised root cultures were established. Out of the two known media *viz.*, White's (1954) and Murashige and Skoog's (1962), the MS liquid medium in its one half strength supported active growth of the excised roots. Both the cytokinins, Kn and BAP, were not necessary for their growth. But auxins improved the growth; IAA at 2 μM/l was optimal for the growth and development of the main root axis which reached to 3.1 cm in length and supported production of 12.6 lateral roots. At the same time, highest biomass of 12.4 mg and 0.37 mg in terms of fresh and dry weights was recorded. IBA at 2 μM/l was found to be superior since the number of laterals increased

to 15, and fresh and dry weights were 14.8 mg and 0.44 mg respectively.

The growth of excised roots was highest in the medium containing sucrose (2%) supplemented with IAA and IBA each at 2 μ M/l where the main root axis ~~reached~~ grew to 4.1 cm with the production of 30 lateral roots. Maximum biomass values of 2012 ± 20 mg and 95 ± 7 mg were recorded in terms of fresh and dry weights respectively.

The growth kinetics of excised roots in culture showed a typical sigmoid curve. These cultured roots accumulated / synthesized 0.001% of tropine at the end of four weeks which increased to 0.002% by the end of eight weeks. Incorporation of an aminoacid precursor L-ornithine in the culture medium enhanced the synthesis/accumulation of tropine by the excised roots. Highest tropine contents of 0.15% was recorded at 20 μ M/l of L-ornithine incorporation after four weeks which ~~reached~~ increased upto 0.35% at the end of eight weeks.

Callus cultures of hypocotyl / leaf explants were established on media supplemented with optimal concentrations of sucrose with Kn and 2,4-D. For hypocotyl callus M S medium with sucrose (2%) supplemented with Kn (1 μ M/l) and 2,4-D (6 μ M/l) supported maximum biomass values (4500 ± 47 mg fresh weight and 225 ± 8 mg dry weights). For leaf callus MS medium containing sucrose (2%) supplemented with Kn (2 μ M/l) in combination with 2,4-D (6 μ M/l) supported highest biomass production (4770 ± 45 mg fresh weight and 240 ± 10 mg dry weight). These media were designated as 'standard media' for this plant.

The hypocotyl / leaf callus tissues showed a normal growth pattern. The leaf callus tissues could accumulate/ synthesize more of tropine alkaloid contents (0.018%) by the end of eight weeks as compared to hypocotyl tissues which could accumulate/ synthesize only 0.001% of tropine. Thus, it was evident that they have retained their inherent biosynthetic potential.

Suspension cultures established from leaf callus tissues accumulated/synthesized maximum of 0.02% of tropine ~~contents~~ by the end of three weeks. Effect of precursor L-ornithine feeding at various concentrations to the suspension cultures showed that maximum ~~of~~ tropine contents of 0.03% were recorded at 20 µ M/l. The cell colonies developed by plating the cell suspension on the standard medium, accumulated/synthesized 0.025% of tropine contents. These colonies after culturing again in the standard medium and replating, showed the presence of tropine ~~contents~~, thus retaining their intrinsic bio-synthetic potential.

Roots were regenerated from leaf callus in presence of IAA whereas in response to lower levels of 2,4-D, the callus turned nodular. Histological observations of the nodular callus revealed meristematic growth centres. In presence of Kn and BAP the nodular callus differentiated into shoot buds. Rooting of the elongated shoots was achieved.

The excised leaf/leaf segments too showed regenerative potential. Profuse roots were regenerated from leaf segments on medium containing IBA (2 µ M/l). Whereas leaf cultured on medium containing Kn and BAP showed a synergestic effect by differentiating adventitious shoot buds.

Haploids were produced from the pollens which were in uninucleate stage of development at the time of inoculation. Diploidised plants were produced by colchicine treatment, where 2n = 24.

The highlights of the present work are as follows :

1. *Withania somnifera* vegetative and fertile plants synthesized/ accumulated tropine alkaloids, highest being in fertile plants.

2. Fertile plants growing in Ellora Park area accumulated highest tropine contents when compared with the plants from various localities. Hence they were selected as 'elite' superior plants.

3. Roots were the site of synthesis/accumulation when compared with various organs such as stem, leaf or fruits.

4. MS liquid medium reduced to half strength containing sucrose (2%), IAA (2 μM/l) and IBA (2 μM/l) supported highest growth of excised roots.

5. Further, the excised roots have showed the capacity to synthesize/ accumulate tropine contents with the feeding of precursor L-ornithine.

6. MS medium with sucrose (2%), supplemented with Kn (1 μM/l) and 2,4-D (6 μM/l) was the standard medium for hypocotyl callus as it supported maximum biomass production. Whereas for leaf callus MS medium with sucrose (2%), supplemented with Kn (2 μM/l) and 2,4-D (6 μM/l) was the standard medium.

7. The biosynthetic capacity of the leaf callus tissues for tropine synthesis was more as compared with hypocotyl callus tissues.

8. Precursor feeding with L-ornithine (20 μM/l) improved tropine synthesis of suspension cultures.

9. High-yielding cell lines were established.

10. Plantlets were regenerated from leaf callus.

11. Leaf/leaf segments of *W. somnifera* regenerated into adventitious buds and roots. Adventitious buds developed into plantlets.

12. MS medium with sucrose (4%) and supplemented with CM (15%) induced haploid plantlets from uninucleate pollens of the third floral buds.

13. Homozygous diploids were produced where 2n = 24.

Further work on 'hairy' root cultures, high-yielding cell lines and haploids needs to be carried out by applying recent technologies.