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EXTERNAL EXAMINER'S EVALUATION REPORT ON THE Ph.D. THESIS ENTITLED, " IN VIVO AND IN VITRO STUDIES ON WITHANIA SOMNIFERA (LINN.) DUNAL ", SUBMITTED BY SMT. ARUNA JOSHI TO THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA.

I. The problem selected for the Thesis concerns the well-known medicinal plant, 'Ashwagandha', which is used for treating certain nerve disorders, and is of potential value as an anti-tumour and stress-reducing agent. The Candidate has employed tissue and cell culture methods to enhance the yield of the tropine alkaloid end-product by adding aminoacid precursors and cell immobilization.

II. The Candidate has used three different sources of the plant material to ascertain the variation in the alkaloid content and in vitro response. Thus, the plants collected from the Ellora Park area of Baroda showed the highest content of tropine (0.55%) on dry weight basis, at the flowering stage. Such 'elite' selections were employed for establishing in vitro root cultures in half-strength MS liquid medium containing sucrose (2%), and auxins (2µM/l each of IAA and IBA). By the incorporation of amino acid precursor L-ornithine, at 20µM/l, the tropine content of roots was enhanced to 0.35% after 8 weeks of culture, showing thereby that the roots are the site of alkaloid biosynthesis/accumulation.

III. On the other hand, the hypocotyl/leaf callus had synthesized 0.018% tropine after 8weeks, and leaf-callus suspension cultures yielded 0.02% tropine after 3 weeks

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in vitro, which got boosted to 0.03% after incorporation of the precursor L-ornithine (20μM/l). Cell-suspension cultures developed from leaf callus on MS medium containing 2% sucrose, 2μM/l Kinetin, and 6μM/l 2,4-D, yielded 0.025% tropine when L-ornithine (10μM/l) was incorporated. The tropine yield could be further enhanced to 0.038% by an increase in precursor level to 30μM/l, and by resuspending the cells afresh and plating.

IV. Organogenesis of shoot-buds was induced in nodular callus of leaf, when Kinetin and BAP levels were increased to 4μM/l each, and especially so when BAP was increased to 4μM/l, giving rise to 15 to 17 buds/explant in 95% cases. Rooting of these buds was achieved by transferring them to MS medium supplemented with 1% sucrose and 1μM/l IBA.

V. To obtain androgenetic haploids, immature anthers excised from the 3rd floral bud were cultured after due surface-sterilization, on MS medium containing coconut-water(10%) + 2% sucrose. After 3 weeks, 70% anthers burst open and pollen embryoids emerged, which on transfer to MS + 4% sucrose + CW 15%, yielded haploid plantlets after 4 weeks. These haploids were diploidized using 0.5% Colchicine for 48 hr, washed and transferred to rooting medium. Chromosome counts revealed 2n=24 in root-tips of these DH plants.

VI. The Discussion chapter is well written, bringing out the significance of the findings in the Thesis, in the light of currently available literature on the subject.

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There was variation in alkaloid content not only due to environmental factors, but also in different plant parts, the roots accumulating maximum (0.25%) in the Ellora Park samples, leaf(0.13%), stem (0.1%), and fruits (0.08%), on dry-weight basis. The Discussion chapter does not indicate whether the cell-suspension culture, or callus system, or the root-culture method would be commercially viable for mass production of the alkaloid on a fermenter scale.

No detailed treatment has been given to the work on Haploids, to say whether the pollen ontogeny of the embryoids was traced, and if so whether it followed the A, B, or C pathway of development as per Sunderland's scheme. Also, the further fate of the diploidized haploids with regard to their alkaloid yields or other economically useful features, have not found a place in the Thesis.

VII. The Summary is crisply written and ends by saying that further work on 'hairy root' cultures is needed, although there is no mention of such 'hairy roots' anywhere else in the Thesis. Also, which of the 'recent technologies' need to be employed on the Haploids and high-yielding cell lines, has not been spelt out clearly.

VIII. The Bibliography leaves much to be desired for want of uniformity in citation, several missing Titles, page-numbers, missing References, that are cited in Text and vice-versa. These omissions and other errors of grammar in the Text as well as in Bibliography listed below, and indicated in pencil

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in the Thesis, should be carefully gone through by the Candidate, and should be corrected and made good, before placing the Thesis in the Library. The following are the References listed in the Bibliography but not cited in the Thesis text:

1. Bhattacharya, S.K. et al. (1987) on p. 108.
2. Brodelius, P. and Nilsson, K. (1980) on p. 109.
3. Majumdar, D.N. (1952) on p. 117.
4. Petri, G. and Bajaj, Y.P.S. (1989) on p. 122.
5. Ross, M.K. and Thorpe, T.A. (1973) is cited as Ross et al. (1973) on p. 99, or is it another Reference ?
6. Balandrin, M.F. and Klocke, J.A. (1988) on p. 107 is cited as Balandrin and Kloche (1988) on p.2; which is the correct spelling of second Author ?
7. Chowdhury, A.R. and Chaturvedi, H.C.(1979) on p. 109 is cited as Chowdhary, and Chaturvedi, H.C., 1979 on p. 17 ? Which is the correct spelling for Chowdhury ?
8. Fairbrain, J.W. and Wassel, (1964) on p. 112 is cited on p. 87 as Fairbrain and Wassol, 1964. Give correct spelling of Wassal/Wassol.
9. Fowler, M.W. (1986a) on p.112; where is 1986b ?
10. Kriegs, W. and Reinhard, E.(1989) on p.116 is cited as Kreis and Reinhard, 1989 on p. 12: Which is correct ?
11. Mothes, K. and Engelbrecht, L. (1956b) on p.120; but where is 1956a ?
12. Tabata, M. and Hiroaka, N. (1976) on p. 127 is cited as Tabata and Hirakoa, 1976 on p. 14. Which is correct ?
13. Weirmann, R.(1978) listed on p.129 is cited as Wiermann, 1978 on pp. 87, 88. Which is the correct spelling ?
14. Zenk et al. (1977a) listed on p.131; where is 1977b ?

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15. Give the Title of Article in the following References: listed in Bibliography : Adams(1979); Alfermann et al.(1989); Arens et al.(1989); Bhattacharya et al.(1987); Butcher and Conolly(1971); Cramer and Turner (1967); Fairbrain and Wassel (1964); Mothes and Engelbrecht (1956); Nowacki(1963); Robins et al.(1987); & Roberts and James (1947).

16. Give the page Nos. in the following References listed in Bibliography : Alfermann et al.(1985); Balandrin and Kloche(1985); Dixon (1985); George and Sherrington (1984); Gusev(1973); Harborne(1972, 1978 & 1982); Jensen (1962); Kinnersley and Dougall(1981); Kitamura(1988); Luckner(1980); Mantel ans Smith (1983); Maretzki et al.(1974); Matveyer(1959); Mitra(1975); Nitzsche and Wenzel (1977); Roberts & James(1947); Shrivastava (1995); Straus (1989); Street and Jones(1963); Szoke et al.(1984); Tabata (1977); Timmermann et al.(1984); Ulbrich et al. (1985); Waddington (1966); Wallace and Mansell (1976); White(1943); and Yasuda et al. (1972);

IX. CONCLUDING REMARKS:

Barring the above-mentioned Errors & Omissions, which should be brought to the attention of the Candidate for effecting the corrections before placing the Thesis in the Library, the research work carried out by her is of high standard & quality.

I have therefore, no hesitation in recommending that the Thesis submitted by Smt. ARUNA GIRISH JOSHI, be accepted for the Award of the Degree of DOCTOR OF PHILOSOPHY in BOTANY of the M.S. UNIVERSITY OF BARODA, VADODARA.

August 1, 1996.

(R.D. IYER)