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The results of experimental work conducted on two medicinally important herbs *viz., Boerhaavia diffusa* L. (Family Nyctaginaceae) and *Achyranthes aspera* L. (Family Amaranthaceae) are summarised in this chapter.

During *in vivo* screening in the ethanolic extract of *B.diffusa* L. plant an alkaloid punarnavine was isolated. Highest punarnavine was accumulated in the roots (2%) of this plant when calculated on dry weight basis. In stem (0.56%) and leaves (0.82%), much lower quantities of punarnavine were isolated. Root samples of *B.diffusa* plants of the same age showed that there was quantitative differences. Highest quantity of punarnavine was isolated from the roots of the plants growing in the M.S. University Campus. Based on the highest quantity of punarnavine in the roots of these plants, 'elite' superior plants were selected and were employed for *in vitro* studies.

The second plant of the present study was *Achyranthes aspera* L. *In vivo* screening of this plants resulted in the isolation of an alkaloid achyranthine (0.11%). In individual organs of a mature *A. aspera* plant showed that the stem contained (0.05%) highest quantity of the achyranthine, while leaves, flowers and roots contained 0.03%, 0.02% and 0.01% of achyranthine respectively. In both these plants, it was indicated that the active principle lies in their alkaloid fractions and hence quantitative estimations were conducted. In *A. aspera* L. plants highest quantity of achyranthine was isolated in Sama area growing plants (0.12%) when

calculated on dry weight basis and hence these plants were identified as 'elite' superior plants.

Since, in *B.diffusa*, roots were the main organs where punarnavine was synthesized/accumulated in maximum quantities, axenic cultures of its excised roots were established. Root tips of aseptically germinated seeds of elite plants were used as explants for the establishment of *in vitro* root cultures.

There are hardly any reports about the excised root cultures of *B.diffusa*. Hence, preliminary work on excised root culture had to be conducted before advanced techniques could be applied for the enhancement of punarnavine by the cultured roots. Out of the known media *viz.*, White's (1954) and Murashige and Skoog's (1962), the MS liquid medium salts supported the active growth and development of excised roots of *B. diffusa*. The incorporation of Kn at 0.5 μM/l enhanced not only the growth of main root axis but also improved the number of lateral roots produced. However, 1 μM/l of Kn was found to be the optimal level, favouring growth of the excised roots in culture and thus it was incorporated in the culture medium.

Out of the auxins IAA/NAA/IBA tested for the enhancement of the growth of the excised roots in culture, IBA at 2 μM/l supported highest growth of the main axis to 17 cm in length with 22 $\pm$ 2, the highest number of lateral roots produced. In case of NAA, the excised roots recorded poor growth and hence it was not used.

The effect of variation in sucrose levels on excised cultured roots of *B.diffusa*

showed that at 2% sucrose, the main root axis recorded highest increase in its dimension (17 cm) with the production of maximum number of lateral roots (22 ± 2).

The salt concentrations present in MS standard medium, when tested indicated that at their standard dose highest biomass production of *B.diffusa* cultured roots was recorded in terms of fresh (790 ± 15.70 mg) and dry weights (55 ± 1.5 mg). Reducing or doubling, the levels of MS medium salts resulted in decline in the fresh and dry weight production of cultured roots.

Even though vitamins are added in minute quantity to root culture medium, they exert profound effect on their growth and development. The levels of vitamins as present in MS medium were studied. Highest growth parameters in terms of the length of the main axis (17 cm) and number of laterals (25) were produced when vitamin levels was as present in the standard MS medium. Lowering or doubling the levels of vitamins of MS medium brought about drastic reduction in these growth parameters.

While studying the growth pattern of excised roots in culture, they showed typical sigmoid curve indicating that it followed the principles of growth in general.

Excised cultured roots also exhibited the ability of alkaloid synthesis which was recorded highest at the end of eight weeks incubation in IBA (2 μM/l) cultured roots (0.06%). The incorporation of amino acid, L-tryptophan in the culture medium improved the synthesis of punarnavine by the roots (0.32%).

Present studies showed that MS medium with sucrose (2%), Kn (1 μM/l) and IBA (2 μM/l) supported the highest growth and development of excised cultured roots of *B. diffusa* and thereby this medium was designated as "standard root culture" medium for this species.

For the callus cultures to be established from stem/leaf segments of *Boerhaavia diffusa* L. required, both Kn and 2,4-D for its induction. The optimal levels of Kn and 2,4-D varied in each case. In *Achyranthes aspera* L. excised stem/leaf segments induced callus in presence of Kn and 2,4-D. No callus induction occurred from floral buds. Highest amount of callus was induced when Kn was 2 μM/l while 2,4-D levels was 4 μM/l. Callus was yellowish in colour and friable. For the maximum biomass production in terms of fresh weight (4449 $\pm$ 59 mg) and dry weights (218 $\pm$ 20 mg) of stem callus of *B.diffusa* required Kn (2 μM/l) and 2,4-D (8 μM/l). While the leaf callus recorded 4104 $\pm$ 29 mg fresh and 205 $\pm$ 16 mg dry weights at the same levels of Kn and 2,4-D. In *A.aspera* stem callus showed highest biomass values (1050 $\pm$ 35 mg, 74 $\pm$ 25 mg) when Kn was 2 μM/l in combination with 4 μM/l of 2,4-D.

As regards the optimal level of sucrose required for both these callus cultures in *B.diffusa* and *A.aspera*, 3% was found to be the optimal dose which supported highest biomass production.

Leaf callus tissues of *B. diffusa* have retained the biosynthetic potential for punarnavine synthesis was seen when it recorded 0.02% at the end of eight week culture period. However, stem callus tissues of *B. diffusa* did not accumulate

appreciable quantity of punarnavine which could be quantified.

A. aspera stem callus synthesized accumulated 0.007% of achyranthine when IAA was incorporated in the culture media. Both these plants callus tissues have retained their biosynthetic potential for punarnavine/achyranthine production.

Histological observations of the stem callus tissues which were became nodular in response to the change in the levels of growth regulators showed growth centres. Before that few of the callus cells developed thickening on their walls in the form of spiral/reticulate form due to lignin deposition. This process was termed as xylogenesis. The growth centres of the nodular callus when provided more dose of cytokinins such as BAP and $AdSO_4$, they produced shoot buds which rooted on transfer to NAA (0.01 μM/l) medium. This clearly indicated that the stem calli, have the regenerative potential and thus plantlets were produced. The rationale behind this was to develop high-yielding cell lines and regenerate plantlets from them but it remained to be done in the present studies.

The regenerative potential of the leaf segments when investigated in *B. diffusa* and in *A.aspera* showed that roots were profusely produced in both of them when cultured on IAA containing medium. These roots originated from phloem parenchyma of the vascular bundle of leaf. In *B.diffusa* these roots after excision could be cultured and they too showed the biosynthetic potential for punarnavine synthesis.

The highlights of the present work are as follows :

1. *Boerhaavia diffusa* L. plants synthesized/accumulated punarnavine alkaloid while those of *Achyranthes aspera* L. achyranthine.

2. Highest quantity of alkaloid accumulation occurred in the roots of *B.diffusa* plants while achyranthine was present in the whole plant as such.

3. Plants growing in the M.S. University Campus of *B.diffusa* accumulted highest punarnavine in their roots when compared with the roots of these plants growing in other localities. Hence they were selected as `elite' plants. In *A. aspera*, Sama area growing plants accumulated maximum achyranthine in them. Hence, they were selected as `elite' superior plants.

4. Excised roots of *B.diffusa* grew well in MS liquid medium.

5. MS liquid medium containing sucrose (2%) Kn (1 μM/l) and IBA (2 μM/l) supported highest growth of *in vitro* grown roots of *B.diffusa*.

6. Vitamins as present in the MS medium supports active growth of excised roots.

7. Addition of L-tryptophan improved synthesis/accumulation of punarnavine by cultured roots of *B.diffusa* L.

8. Stem/leaf of *B.diffusa* and *A.aspera* induced maximum callus in presence of sucrose (2%), Kn (0.5/2 μM/l) and 2,4-D (2/4 μM/l) respetively.

9. Stem Callus showed differentiation of tracheary elements in presence of low levels of 2,4-D in the medium.

10. Callus showed morphogenic response and produced nodules consisting meristematic cells arranged in a compact mass.

11 Nodular callus developed shoot buds in presence of high dose of cytokinin.

12 Each shoot bud on separation and culturing produced a plantlet in both the plants under study.

13 Leaf callus of *B.diffusa* possessed biosynthetic capacity for punarnavine synthesis while stem callus of *A.aspera* had produced achyranthine.

14 In *B.diffusa* L. and *A. aspera* L., roots were regenerated from the excised leaf segments in presence of IAA in the culture medium.

15 Regenerated roots of *B.diffusa* possessed biosynthetic potential for punarnavine synthesis.

These results clearly supported that both these plants possessed excellent biosynthetic potential for secondary metabolite synthesis which needs further exploration using advanced research technologies.