BIOTECHNOLOGY-A NOVEL TECHNIQUE FOR CONSERVATION OF ARID REGION PLANTS

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Abstract

Rapid and high frequency in vitro multiple shoots and subsequent plantlets were developed in several plant species of arid forestry viz. Anogeissus acuminata, A.pendula, A. latifolia, A.sericea var. nummularia, A.sericea var. sericea, Calligonum polygonoides and Withania coagulans. In Prosopis cineraria, Tecomella undulata, Acacia senegal, Maytenus emerginata, Balanites aegyptiaca, Zizyphus nummularia and Z. mauritiana in vitro multiple shoots were induced at the high rate by using nodal shoot segments from mature trees, but root induction in differentiated shoots were not very high in few species. Dipcadi erythraeum - an endemic and endangered plant is differentiated into plantlet from in vitro cultures.

Various explants (cotyledonary nodal segment and epicotyl of Anogeissus spp., nodal and apical shoot segments from mature plants) of 1.5-2.5 cm in length were placed on Murashige and Skoog's 1962(MS) basal medium supplemented with inorganic and organic addenda. Various growth regulators (auxins IAA, IBA, NAA and 2, 4-D and cytokinins; kinetin and BAP) were added from 0.1- 5.0 mg/l either alone or in combination to induce multiple shoots. Differentiated shoots could be further multiplied on fresh medium. Isolated shoots were rooted on MS basal and MS half strength basal salts medium with various concentrations of IAA, IBA and NAA.

Plantlets of Anogeissus spp., Calligonum polygonoides, Withania coagulans and Dipcadi erythraeum were transferred into pots. . (N. S. Shekhawat et al. 1993,Rathore et al 1991,kaur et al 1992)

INTRODUCTION

The economy of the people of Rajasthan is greatly dependent on the forest wealth in many ways for fuel, food, fodder, timber, drugs and other commercial products. The pressure of growing population has however caused the over-exploitation of the forests to a disastrous proportion. A large scale plantation of priority plants for afforestation of degraded forest and wasteland could be the solution to ensure future sufficiency in biomass.

Plant biotechnology has opened up the door to (1) rapid clonal multiplication of genetically uniform plants from the elite material, (2) the selection of novel and improved varieties using somaclonal variation, (3) the development of new hybrid by means of protoplast fusion, (4) production of virus free plants by meristem culture, (5) the production of biological active compounds for pharmaceutical industry, (6) maintaining viability of valuable germplasm over a number of years by cryopreservation and (7) the use of recombinant DNA to introduce new genetic material into plant cells to develop drought, cold, salts, disease, pests and herbicide resistant plants.

Commercial application of forest trees tissue culture is presently limited to micropropagation of Poplars, Red-wood, Sequoia, Eucalyptus, Radiata pine, Sandal wood, teak, bamboos and some other species (Mascarenhas et al., 1981; Biondi and Thorpe, 1981; Lakshmi Sita et al., 1982; Thorpe and Biondi, 1984; Powledge, 1984; Haissig et al., 1987; Radojevic, 1988; Nadguada et al., 1990). The work done in our country

on biotechnology as Related to arid zone forestry has been reviewed extensively (Mascarenhas and Muralidharan, 1989; Shekhawat and Johri, 1991).

This paper deals with the micropropagation of some important forest plants of semi-arid and arid regions of Rajasthan. The plant specics included are Anogeissus acuminata, A.pendula, A. latifolia, A.sericea var. nummularia, A.sericea var. sericea, Acacia senegal, Balanites aegyptiaca, Calligonum polygonoides, Dipcadi erythraeum, Maytenus emarginata, Prosopis cineraria, Tecomella undulata, Withania coagulans, Ziziphus nummularia and Z.mauritiana. These plants are important source of fuel, fodder, vegetables, biomass, timber and medicine etc. In addition these species are important component of desert ecosystem and have impact on biological life in many ways.

Prosopis cineraria, Tecomella undulata, Maytenus emarginata, Acacia senegal, and Ziziphus spp. are highly cross pollinated. Therefore, selected and tested plants were used for clonal multiplication. Anogeissus spp., Calligonum polygonoides, Withania coagulans are slow growing and natural propagation is mainly by seeds with very low percentage of seed germination and high rate of seedling mortality. Therefore these plants were selected for micropropagation using various explants.

Materials and Methods

Fruits of Anogeissus acuminata, A.pendula, A.latifolia, A.sericea var. nummularia, A.sericea var. sericea and Acacia senegal were collected from Mount Abu, Ranakpur, Udaipur, Kailana (Jodhpur) and Supka (Nagaur). Fruits wall were removed mechanically and viable seeds were inoculated aseptically on MS medium (Murashige and Skoog, 1962). Seedlings were used a source of explants in Anogeissus spp. and A.senegal. Explants (axillary bud, apical bud and nodal shoot segment) were taken from mature plants in Prosopis cineraria, Tecomella undulata, Calligonum polygonoides, Maytenus emarginata, Balanites aegyptiaca, Ziziphus spp. and Withania coagulans. Surface sterilized explants were cut into segments and placed aseptically on medium. Cultures were incubated at 26± 2°C temperature, 2000- 3000 lux intensity of light for 12 h photoperiod and 60-65% relative humidity. Various media were tested to select appropriate medium for the establishment and multiplication of cultures. Auxins viz., IAA, IBA, NAA and 2, 4-D and Cytokinins; kinetin and BAP were added in the media from 0.1-5.0 mg/l either alone or in combinations for the induction of callus, its differentiation, direct multiple shoot induction, multiplication and root induction. Arginine (10-50 mg/l), adenine sulphate (10-15 mg/l) were used in culture medium for P.cineraria, Tundulata, A.acuminata and A.sericea. Yeast extract (500 mg/l) and coconut milk 10-15% were supplemented in the MS medium to develop cultures of Dipcadi erythraeum. Agar (0.6-0.8%) gelling agent added in the media.

Multiple shoots induced in Anogeissus spp. from cotyledonary nodal segment and epicotyl were subcultured for further multiplication on fresh MS medium with IAA (0.1 mg/l) + BAP (0.5-2.5 mg/l). Differentiated shoots were subcultured on root induction medium (MS half strength + IBA, 0.5-2.0 mg/l). P.cineraria, T.undulata, M.emarginata, B.aegyptiaca, Ziziphus spp. and W.coagulans multiple shoots induced on MS + IAA 0.01-0.1 mg/l + BAP 0.5-5.0 mg/1 medium were subcultured for further multiplication on comparative lower cytokinin medium (BAP 0.25-2.5 mg/l).

Isolated shoots were inoculated on half strength basal salts of MS medium with IBA / NAA at various concentrations (0.5-2.5 mg/l) alone or with Kn (0.1-0.5 mg/l) for root induction. Callus cultures of Dicadi were raised from bulb, scale and leaf segments on MS medium supplemented with 2, 4-D coconut milk/NAA + BAP. Callus differentiating medium was MS + BAP / MS + NAA + BAP). Direct shoot primordias induced on MS + IAA + NAA + higher BAP. Differentiated shoots of Dipcadi were subcultured for root induction on MS full and half strength basal salts with IBA / NAA.

Plantlets with well developed root and shoot were carefully taken out from the culture vessels and washed thoroughly. Finally plantlets were transferred into earthen and plastic pots containing various soil mixtures with or without varmiculite. Pots were covered by polythene bags to check the excess loss of water. Plants were hardened under semicontrolled conditions.

Results and Discussions

Out of the various media used MS basal medium was found the most ideal for the establishment, differentiation and multiplication of cultures for all the plant species. Micropropagation of all the plant species undertaken were obtained in three steps: (i) Multiple shoot induction from the explant used, (ii) Further multiplication of shoots from subcultured shoot or original explant, and (iii) Root induction from isolated shoots and subsequent transfer into pots.

Cotyledonary nodal shoot segment was found the best explant for the multiple shoot induction in Anogeissus spp. and Acacia senegal whereas nodal shoots segment were found most idcal in Prosopis cineraria, Tecomella undulata, Calligonum polygonoides, Maytenus emarginata, Balanites aegyptiaca, Withania coagulans, Ziziphus nummularia and Z.mauritiana.

Anogeissus acuminata-multiple shoots (20-25 shoots/culture) were obtained on MS medium supplemented with IAA 0.1 mg/1 + BAP 1.0-2.5 mg/l within 4-5 weeks. Subcultured shoots further multiplied the best on this medium (MS + IAA 0.1 + BAP 0.5-1.5 mg/l). Differentiated shoots rooted on half strength MS salts supplemented with IBA (0.5-1.5 mg/l) within 3-4 weeks. Root induction was achieved in 90-95% shoots. A.pendula cotyledonary nodal segment induced 15-20 shoots on MS medium incorporated with IAA (0.1 mg/l) + BAP 1-2.5 mg/l. Further multiplication of shoots was obtained on MS medium with lower strength BAP (0.5-1.0 mg/l). On half strength MS medium with IBA (1.5 mg/l) + Kn (0.1 mg/l) the shoots rooted.

A.latifolia- multiple shoots were obtained from cotyledonary nodal segment as well as from the epicotyl segments on shoot induction medium (MS + IAA 0.1 mg/l) + BAP (1.5-2.5 mg/l) but frequency of shoot induction was less from epicotyl (3- 4 shoots) as compared to cotyledonary (15-20 shoots). Subcultured shoot could be further multiplied on fresh medium. Differentiated shoot rooted while subculturing on hormone free MS & half strength MS medium but addition of IBA in the medium induced strong and viable roots.

A.sericea var.nummularia-cotyledonary nodal segment induced 10- 12 shoots on shoot induction medium (MS+ IAA 0.1 + BAP 2.0-3.0 mg/l) within 3-4 weeks. Original explant induced enhanced multiple shoots (15-20) upto 5 passages on fresh shoot induction medium. Subcultured shoots rooted the best on MS half strength basal salts medium with IBA / NAA within 3-4 weeks.

A.sericea var. sericea - ascorbic acid, citric acid and adenine sulphate were added in the MS medium. Requirement of cytokinin (BAP) was high as compared to other species of Anogeissus. Maximum 10-12 shoots were induced from cotyledonary nodal segment on MS medium + IAA + BAP (2.5-3.5 mg/l) +addenda. Subcultured shoots multiplied on the fresh shoot induction medium. Original explant could be subcultured upto 5-6 successive culture for multiple shoot induction. Incorporation of IAA / IBA in the MS half strength medium induced roots in the subcultured shoots.

Acacia senegal - shoots were induced on the MS basal medium + IAA + BAP + Addenda within 4-5 weeks. Isolated shoots could be further multiplied on the same fresh medium. Subcultured shoots rooted on MS half strength basal medium with IBA + NAA but percentage root induction was less than Anogeissus spp.

Prosopis cineraria - nodal shoot segment induced 10-12 shoots on MS medium with IAA 0.1 mg/1 + BAP 2-5 mg/1 + addenda within 3-4 weeks. Original explant could be used upto 4-5 successive cultures for the multiple shoot induction in the fresh shoot induction medium with low BAP 1.0 mg/l. Sporadic root induction observed in the subcultured shoot on MS + NAA + Kn/MS + IBA + Kn medium.

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Tecomella undulata apical and nodal shoot segments induced multiple shoots on MS medium IAA (0.01-0.1 mg/l) + BAP 1.5-2.5 mg/l within 3-4 weeks. Subcultured shoot further multiplied on lower BAP medium (MS + IAA 0.01 mg/1 + BAP 0.5-1.0 mg/l). Root induction was observed from the cultures which were kept on MS liquid medium + IBA (10-25 mg/l) with filter paper bridge for 24 hr followed by subculture on hormone free medium.

Maytenus emarginata - adventitious shoots were induced from the nodal shoot segment on MS medium supplemented with IAA + NAA + BAP within 4-5 weeks. Shoot primordias were subcultured and multiplied best on MS + IAA + Kn (0.5-1.0 mg/l)/MS + IAA + BAP (0.25-0.5 mg/l). Multiplication rate was very high (60-80 shoots per culture) in this species.

Ziziphus nummularia - nodal shoot segments found better than apical shoot buds for multiple shoot induction. Combined Kn + BAP proved useful along with IAA for the multiple shoots. Original explant and subcultured shoots could be further multiplied on MS + IAA 0.01 mg/l + BAP 1.5 mg/l + Kn 1.5 mg/l. Isolated shoots rooted on MS medium with IAA + IBA + NAA within 4 weeks.

Calligonum polygonoides - multiple shoots were regenerated from explants within a week. Each explant produced 18-20 shoot buds. When these shoot-buds were attained a length of 1-2 cms these were subcultured on MS + BAP (0.5 mg/l) + NAA (0.1 mg/l). On this medium 35-40 shoots from each shoot bud were proliferated within one week. After 4 weeks when these shoots became 2-3 cms they were transferred on rooting medium (IBA 0.5 mg/1 + NAA 0.1 mg/l). About 60 shoots were rooted on this medium within a period of 2 weeks.

Balanites aegyptiaca - multiple shoots were regenerated from mature explants within 9-10 days. Each explant produced 5 to 6 shoots. When these shoots were attained a length of 3-4 cm they were sub-cultured on MS + BAP (0.5 mg/l) and IAA (0.01 mg/l) on this medium 9 to 11 shoots were proliferated within a week.

Withania coagulans - callus was induced on MS medium with 2,4-D + Kn/NAA + Kn from leaf and stem segments. Shoots were induced from the nodal shoot segment on MS + IAA + BAP medium. Further multiplication of shoots was obtained on MS medium + IAA + BAP + GA3. Viable roots were induced in subcultured shoots on MS half strength medium with supplemented with IAA / IBA (0.5-1.0 mg/l) within 3-4 weeks.

Dipcadi erythraeum - direct multiple shoot induction was achieved from bulb explant. Scale segments differentiated into shoots via callus on MS medium + 2,4-D + coconut milk, Leaf tissues developed adventitious buds on MS medium + IAA + NAA + BAP. Isolated shoots rooted on half strength MS medium incorporated with IAA / NAA / IBA within 4-5 weeks.

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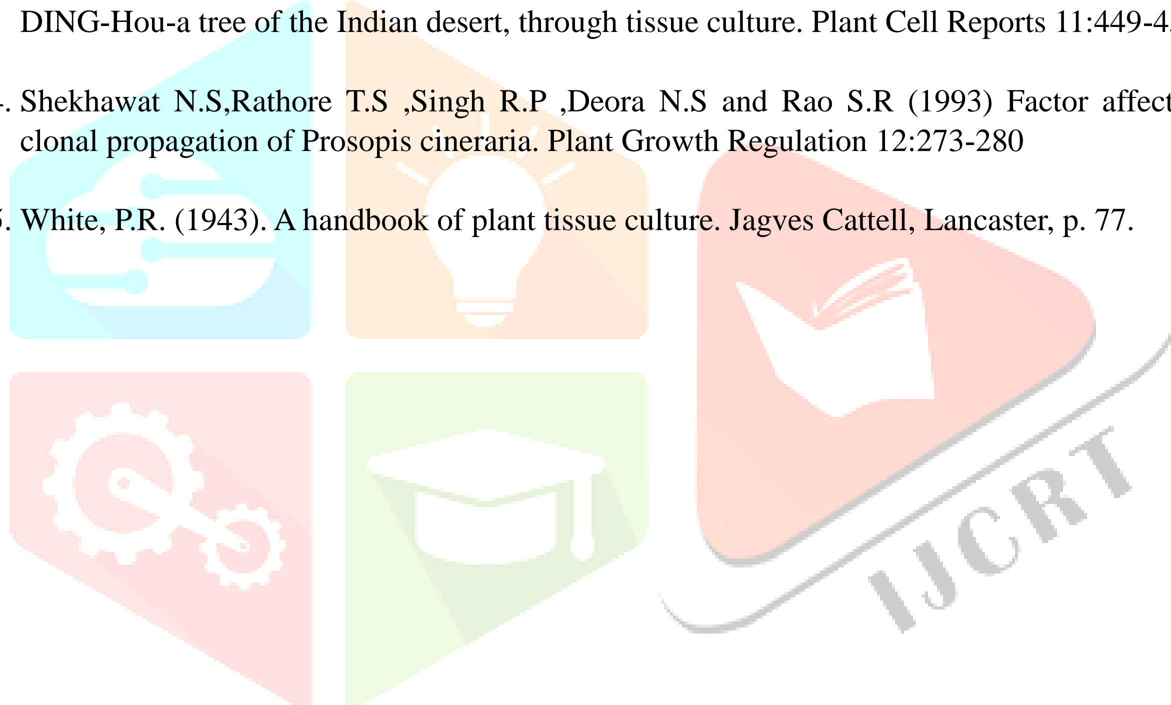

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