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RAPID MICROPROPAGATION OF PLANTS OF ARID REGION

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ABSTRACT

Plant tissue culture methods were applied for micropropagation of Anogeissus latifolia, Caraluma edulis, Ceropegia bulbosa and Withania coagulans. These are ecologically and economically valuable plant species of fragile ecosystems of the Aravalis and the Indian desert. Micropropagation protocol was developed for Anogeissus acuminata – a tree of the Aravalis. Cotyledonary node explants were cultured on MS + 2.0 mgl-1 BAP + 0.1 mgl-1 NAA and other additives. The shoots were multiplied for large scale plant regeneration. These were rooted on half strength MS salts + 0.1 mgl-1 IBA + 100 mgl-1 activated charcoal. Tissue culture processes were also defined for C. edulis and C. bulbosa. In case of withania coagulans cultures were established and multiplied on modified MS medium. Plantlets generated through tissue culture were hardened in green house and transferred to pots/polybags. The procedure developed can be useful for large scale production of these plants for afforestation and also for the conservation of germplasm.

Keywords: *Anogenissis Acuminata, Ceropegia Bulbosa, Withania Coagulans, Micropropagation.*

Introduction

Plant tissue culture techniques are circumventing some of the obstacles of traditional breeding. Tissue culture has largely been integrated in biotechnology that permits the regeneration of plants as clones and as transgenics. In commercial production, plant tissue culture emerges as an advantageous means of mass propagating economically valuable clones and new crop introductions, with concurrent gains in production timing, product uniformity, efficiency and availability of clean, virus-free material and flexibility in response to market demands. In research, in vitro technique facilitates the engineering and selection of elite, superior genotypes, and serves as a vehical for in-depth investigation of physiological or biochemical processes (Smith, 1994). Among the many tissue culture technologies, micropropation is one of the most powerful and widely used (Zimmerman, 1996).

The Indian Thar desert and the neighbouring Aravalis are two ecologically fragile systems which harbor germplasms of several plant species that can withstand different types of stresses. As part of our programme on development of tissue culture processes for conservation and propagation of germplasm of some of the plants of these regions we describe here. Methods devolped for micropropagation of Anogeissus acuminata, Caralluma edulis, Ceregegia bulbosa and Withania coagulans.

Anogeisus spp. – a member of combretaceae, is an important tree of forestry. This tree is included in the list of silk producing non-mulberry plants (Srivastava et al., 1990) that also yields a gum which substitutes for Gum-Arabica and is constituent of **gum-ghatti** of commerce. The tree produces tough wood used for making axels, shafts, poles, batons and tool-handles. Extremely poor seed viability, poor propagation in nature and the overuse has resulted in the decline in population of this plant. Caralluma edulis (asclepiadeaceae) is leafless plant with fleshy succulent shoots. This is and edible plant of extreme xeric environment of Jaislmer. Locally it is known as Pimpa. The shoots of this plants are chopped and cooked as vegetable (Arora abd Pandey 1996). This is rare plant of western Rajasthan (Pandey et al., 1982). Ceropegia bulbosa (Khedula) is another edible member of asclepiadaceae and is

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threatened in is its habitat. This plant contains ceropegine-an alkaloid. The tubers are considered tonic and used for treatment of gastric disorders (Asolkar et al., 1992; Chopra et al., 1992). Withania coagulans (Paneer Bandh, Akri) is a rare solanaceous plant of arid zones (Bhandari, 1990). This plant has medicinal values and is used in Ayurvedic and Unani systems (Gaind and Budhiraja, 1966).

Material and Methods

Fruits of Anogeissus acuminata were collected from Mount Abu (Sirohi), Ranak Pur (Pali and Udaipur) and Parasram Mahadeo (Pali and Rajasmand) areas. Viable seeds were selected, surface sterilized and washed thouroughly with sterile water. The surface sterilized seeds were germinated aseptically on half-strength hormone-free MS (Murashige and Skoog, 1962) medium containing 500 mgl^{-1} AC. Cotyledonary node of 4-weeks-old seedlings were used as a source of explants. These were placed onto MS medium containing cytokinins, auxins and other additives. Plants of Caralluma edulis were collected from areas nearer to Jaislmer with the help of Bheel Shepherd boys. These were transported to Jodhpur and maintained in the green house. Shoot apices and nodal shoot segments were used as explants after surface sterilization. The explants were cultured on MS medium containing auxins and cytokinins.

Field surveys were conducted during rainy season to locate plants of Ceropegia bulbosa in the Thar desert. A few plants were found on very old and highly protected field boundries in Sikar and Jhunjhunu districts. Some of these plants were uprooted and transported to lab and maintained in the green house. Fresh shoot sprouts were harvested and surface sterilized. Nodal shoot explants and apical shoots were cultured on MS medium containing BAP + auxins.

Fresh and healthy shoots of Withania coagulans were collected from Chohatan (Barmer). These were transported to lab. Nodal stem sections (3-5 cm) were surface sterilized with mercuric chloride (0.1-0.2%) for 5-10 minutes, washed thoroughly with sterile water and inoculated on culture medium. Alternatively, small plants were transferred to pots alongwith root stock and habitat soil and maintained in the green house. Once these were harvested and washed with autoclaved and chilled water containing ascorbic acid (100 mgl^{-1}). These were surface sterilized with mercuric chloride (0.1%) for 3-4 minutes and washed several times sterile water. The nodal segments were cultured on MS medium containing ascorbic acid, citric acid and L-asparagine and L-glutamine. A combination of BAP (2.0 mgl^{-1}), Kintetin (5.0 mgl^{-1}) and 0.5 mgl^{-1} each of GA_3, IAA and NAA were used as plant growth regulators. 25 mgl^{-1} each of adenine sulphate, arginine and citric acid added in the medium as additives.

Once the cultures were established, these were further multiplied by (a) subculturing of nodal segments of the in vitro generated shoots and (b) repeated transfer of mother epxlants and shoot clusters produced in vitro on MS medium. However, to check vitrification the shoots of W. coagulans were multiplied on modified MS salts (500 mgl^{-1} each of KNO_3 and NH_4NO_3 with) with 0.5 mgl^{-1} each of BAP, Kinetin NAA and GA_3.

The shoots of the plant species produced in culture were rooted by (a) culturing on ½ or ¼ strength of MS salts containing IBA/NAA or (b) by pulsing of shoots with solution of 100,200,400,500 mgl^{-1} of IBA and then culturing on ¼ strength MS medium at 30±2° C.

The plantlets generated in culture were hardened in green house by gradual exposure to decreasing humidity and rising temperatures. These were transferred first to bottles containing soilrite and after 3-4 weeks to polybags having habitat soil.

Results and Discussion

Multiple Shoots of Dhawk (Anogeissus acuminata), were intiated from seedling- derived cotyledonary node explants. The shoots differentiated from cotyledonary node on MS + 2.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA or IAA + additives. Shoot cultures of C. edulis were initiated from cultured nodal shoot segments on MS + 2.5 mgl^{-1} BAP. Four to six shoots differentiated from each node of the explants. Cultures of C. bulbosa were initiated from nodal shoot explants on MS supplemented with 3.0 to 5.0 mgl^{-1} of BAP + 0.1 mgl^{-1} of NAA and 25 mgl^{-1} each of the adenine sulphate, argnine, citric acid and 50 mgl^{-1} of ascorbic acid. About 4-7 shoots were differentiated from each node.

Withania coagulans is a very rare plant. Few plants were found neat Chohtan (Barmer), and a few in Jodhpur. About 12-15 plants are surviving near Chohtan some of these bear tiny flowers. The plants materials collected from the field proved difficult to establish in cultures as these carry recalcitrant microbes and it was almost impossible to get the cultures contamination-free. The fresh shoots regenerated from pruned plants proved to be suitable source of explants for the establishment of cultures. The browning of explants could be checked by keeping the surface sterilized explants in

autoclaved chilled water containing ascorbic acid and citric acids. After 10-15 days of the culture, bud breaking occurred and one or two shoots appeared from each node of W. coagulans. The best medium for explant culture and bud breaking was MS containing 50 mgl^{-1} each of ascorbic and citric acids, 1 mM each of asparagine and glutamine and combination BAP (2.0 mgl^{-1}), 0.5 mgl^{-1} each of Kinetin, GA_3, or NAA and adenine sulphate (25 mgl^{-1}).

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Abbreviation

AC, Activated Charcoal; BAP, Benzylaminopurine; GA_3. Gibberellic acid; IAA, Indoleacetic acid; IBA, Indolebutyric acid; NAA, Naphthalene acetic acid; MS, Murashige and Shoog's medium.