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SHORT COMMUNICATION

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Biotechnological approaches for propagation and prospecting of important medicinal plants from Indian Thar Desert

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Abstract

The "Thar Desert" region of Rajasthan in north-west India is one of the most inhospitable landscapes on earth. The desert has sparse vegetation and the ground surface is exposed to sun, atmosphere and associated physical forces. Ever increasing human and livestock population have put heavy demands for plant products, resulting in over exploitation of well adapted plants. Plants like species of *Ceropegia*, *Caralluma, Calotropis, Leptadenia, Tylophora, Pueraria, Mucuna, Vitex and Sarcostemma* are valuable sources of fuel, fodder, timber, medicine, biofertilizer (Endophytic rizobia) and vegetables for sustaining life in the desert. These plants are well adapted to the environment and contribute to the productivity, and keep the system photosynthetically active under all seasons. Attempt has also been made beside tissue culture, to isolate and purify the rhizobacteria associated with important native legumes of this region. These studies are important for sustainable agriculture practices.

Keywords: *Leptadenia*; *Mucuna pruriens*; *Pueraria tuberosa*; Rhizobia; *Tylophora*

Introduction

The north-west region of Rajasthan is known as "Western Sandy Plain". The remarkable feature of Rajasthan is Aravalli range, the oldest folded mountain range in the world. This rocky and hilly area occupies important position in the system as it probably affects movement of monsoon and influences the entire ecology. The scenario is changing due to large scale deforestation of the area. Water is scarce, and non availability of water is a major constraint (Bhandari, 1990). Plants with xerophytic adaptation are able to survive and evolve. As a part of survival mechanisms the plant species produce numerous metabolites. Some of these metabolites are used as pharmaceuticals, agrochemicals, flavors and ingredients of fragrance, food additives etc. Increasing human population and developmental activities have caused irreversible damage to the natural habitats and reproduction cycle of numerous plant species in the Indian Thar Desert and the Aravallies

(Rathore et al., 2009). Hence, information of plant species became an important for plant sourcing (Goodger et al., 2008). Plants species (*Ceropegia*, *Caralluma, Calotropis, Leptadenia, Tylophora, Pueraria, Mucuna, Vitex and Sarcostemma*) are of great importance as per sustainable utilization. Some of these are either edible, wild legumes (*Puraria tuberosa* and *Mucuna pruriens*) harbor important rhizobacteria or yield products which are valuable sources of medicines. However, increasing habitat disturbances, over-exploitation and slow reproduction and need of appropriate pollinator, limit population of these plant species (Rathore et al., 2009).

There is an urgent need for application of conventional and non-conventional methods for conservation of germplasm and for propagation for sustainable utilization (Rathore et al., 2009). By employing such methods large number of plants can be produced starting from a single individual in a relatively short time (Bhojwani and Razdan, 1996).

Materials and Methods

Explants of *Ceropegia bulbosa*, *Leptadenia reticulata*, *Caralluma edulis*, *Sarcostemma acidum, Vitex negundo, Mucuna pruriens, Pueraria tuberosa* and *Tylophora indica* were harvested from various sites of Rajasthan (also from Aravallies). Nodal shoot segments were used directly as source of explants. Prior to surface sterilization explants were pretreated with Bavistin and 0.01% tetracycline for 15-20 minutes. These explants were then surface sterilized with 0.1% $HgCl_2$ for 3-5 min. followed by a dip in 90% ethanol for 30 seconds. The explants were inoculated vertically on agar-gelled MS (Murashige and Skoog, 1962) medium containing plant growth regulators (Benzylaminopurine, BAP or Kinetin) of varying concentrations (0.5 mgl^{-1} -0.5 mgl^{-1}) in culture tubes and were then incubated in growth chambers at 27±2 °C, 60% relative humidity, 30-40 $\mu molm^{-2}s^{-1}$ SFP for 12-14 hr d^{-1} photoperiod. Multiple shoots regenerated in cultures after repeated subculturing. Regenerated shoots were treated with root inducing PGRs (Indolebutyric acid, IBA or Naphthaleneacetic acid NAA or Naphthoxyacetic acid, NOA alone or in different combinations). The auxin treated individual shoots were rooted by *in vitro* and *ex vitro* approaches. Initially for a week, the bottles were capped with polycarbonate caps and placed near pad section of green house (80% relative Humidity and temperature 26±2 °C) subsequently the caps were loosened and these were removed after 20-25 days. The hardened plants were transferred to polybags containing soil, organic manure and vermicompost in 3:1:1 ratio. After 3-4 weeks these were transferred to nursery. Rhizobacteria were isolated from root sterilized nodules present on the secondary roots. These were cultured on CR-YEMA medium and maintained in incubator at 28±2 °C temperature. Pure cultures were prepared by serial dilution.

Results

Cultures of *L. reticulata* were established on MS semi-solid medium supplemented with 6- benzyl adenine (BAP, 1.0 mgl^{-1}) and ammonium sulphate 125.0 mgl^{-1} (Figure 1A) (Table 1). Shoots produced were rooted both by *in vitro* and *ex vitro* approaches and were transferred to fields. Seedling-derived explants were used for culture initiation in *Ceropegia bulbosa*. On MS 1.0 mgl^{-1} of 2, 4-D, the epicotyls produced regenerative cell cultures. Cultures differentiated with high frequency through organogenesis into multiple shoots

(Table 2). The shoots produced in culture rooted *ex vitro* after treatment with 100.0 mgl^{-1} of IBA. Explants of *Caralluma* showed optimum results on MS +3.0 mgl^{-1} BAP (Table 3, Figure 1B). The *in vitro* produced shoots were excised and inoculated on ¼ strength of MS medium +0.1% activated charcoal +5.0 mgl^{-1} of IBA found to be optimum for root induction. Maximum response (80%) was recorded. After treatment with 300.0 mgl^{-1} of IBA 80 percent of the shoots were rooted under *ex vitro* conditions. Establishment of cell cultures for *Sarcostemma acidum* was achieved on MS medium supplemented with 2.0 mgl^{-1} of 2, 4-D Cultures were further multiplied on low concentration of 2,4-D (0.5 mgl^{-1}) and BAP (0.25 mgl^{-1}). In *Tylophora indica* multiple shoots differentiated from the nodal explants on MS +2.0 mgl^{-1} of BAP. About 70.0% of the explants produced multiple shoots within 5-7 days of inoculation. We have developed micropropagation protocols of *Pueraria tuberosa, Vitex negundo* and *Mucuna pruriens*. These plants were successfully hardened and acclimatized. These plants have limited germplasm. *P. tuberosa* and *M. pruriens* are important native legumes which have now a day's gained importance for their nitrogen fixation abilities. These plants harbor endophytic bacteria which can be utilized for plant growth promoting (PGP) activities and also for induced systemic tolerance (IST) (Yang et al., 2009). Ten rhizobial isolates were extracted from nodules of *Pueraria tuberosa* in pure form and these were assigned as JNVU/PT1-JNVU/PT10. In *Mucuna pruriens* a total of 17 isolates were prepared and were designated as JNVU/M1-JNVU/M17. Isolates are under experimentation for their PGP activities as well as for their molecular characterization (Figure 1C).

Table 1. Effects of different cytokinins on multiplication of *L. reticulata* on MS medium + additives.

PGR concentration (mg/l)		Shoot number ± SD	Shoot length ± SD (cm)
Control		1.4±0.51	1.24±0.14
BAP			
	0.25	3.5±0.52	3.80±0.36
	0.5	3.7±0.67	4.85±0.38
	1.0	5.2±0.63	6.05±0.37
	1.5	4.1±0.73	4.98±0.20
	2.0	3.1±0.73	4.02±0.24
Kinetin			
	0.25	2.1±0.87	1.76±0.43
	0.5	2.4±0.69	2.08±0.28
	1.0	2.7±0.67	3.37±0.29
	1.5	3.0±0.81	3.94±0.39
	2.0	1.7±0.82	2.70±0.38
Computed F			
BAP			
	Replication	1.086955^{ns}	0.6246473^{ns}
	Treatment	38.44559**	278.7189**
	CD	0.577942	0.2802221
Kinetin			
	Replication	0.7024764^{ns}	1.37609^{ns}
	Treatment	6.276537**	96.38411**
	CD	0.6898646	0.2963394

^{ns}Non-significant; *significant ($p \leq 0.05$); **highly significant ($p \leq 0.01$).

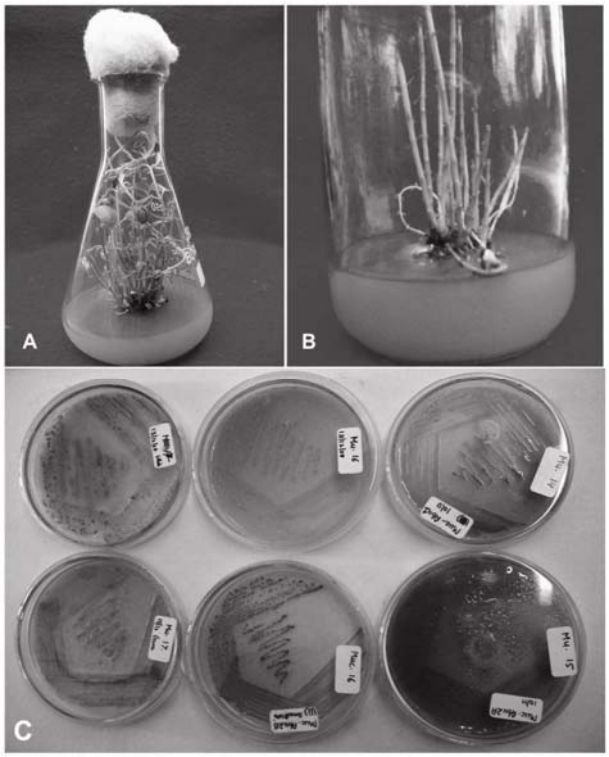

Figure 1.
Figure1 A. *In vitro* amplified shoots of *L. reticulata*.
Figure 1 B. Amplified shoots of *C. edulis* in culture.
Figure 1 C. Pure rhizobial isolates from *M. pruriens.*

Table 2. Effect of concentrations of BAP along with +0.1 mgl^{-1} NAA on regeneration of callus of *Ceropegia bulbosa*.

Concentration of PGR (mgl^{-1})	Response	Remarks
BAP		
0.1	+	Green to yellow and slow growing callus.
0.5	+	Callus green to off white in color
1.0	+ +	Hard, compact, green and slow growing callus
1.5	+ +	Hard, compact, yellow to green moderately growing callus
2.0	+ + +	Globular, fragile with yellowish green color callus
3.0	+	Globular, green, fragile and regenerative callus

+ = slow growing; ++ = moderately growing; +++ = fast growing.

Table 3. Effect of different concentrations of cytokinins on multiplication of shoots in *Caralluma edulis*.

PGR concentration (mg/l)		Response (%)	Shoot number ± SD	Shoot length ± SD (cm)
Control		0	0.1±0.31	0.13±0.41
BAP				
	0.5	55	1.2±0.42	2.06±0.94
	1.0	75	1.6±0.51	3.85±0.47
	2.0	95	2.4±0.84	5.48±0.43
	3.0	95	1.8±0.63	3.62±0.62
	4.0	85	1.7±0.48	3.13±0.56
	5.0	85	1.5±0.52	2.65±0.51
Kinetin	0.5	40	0.4±0.69	0.44±0.72
	1.0	55	1.1±0.73	1.38±0.36
	2.0	65	1.6±0.51	2.19±0.32
	3.0	75	2.1±0.87	2.69±0.91
	4.0	85	2.4±0.51	4.05±0.47
	5.0	80	1.9±0.56	3.22±0.34
Computed F				
BAP				
	Replication		1.69737^{ns}	0.6383548^{ns}
	Treatment		17.72933^{**}	74.32257^{**}
	CD		0.4745368	0.5443335
Kinetin				
	Replication		0.3823497^{ns}	1.682405^{ns}
	Treatment		17.58087^{**}	72.3153^{**}
	CD		0.5877049	0.4811332

^{ns}Non-significant; *significant ($p \leq 0.05$); **highly significant ($p \leq 0.01$).

Discussion

A typical feature of plant development is the wide role played by cell-cell signaling in regulating patterns of growth and cell fortune (Golz, 2006). Cytokinin treatments are recommended for micropropagation of woody trees (Aitken-Christie and Connett, 1992; Rathore et al., 1993; Shekhawat et al., 1993). Among the cytokinins BAP was found superior to Kinetin (Bonga and Von Aderkas, 1992). Amplification of shoots was achieved by repeated transfer of explants, has been reported to be useful for cloning of adult trees (Boulay, 1987; Franclet et al., 1989; Deora and Shekhawat, 1995). Induction of rooting is affected by several intrinsic and extrinsic factors (Wilson and Van Staden, 1990; Schiefelbein and Benfey, 1991). The auxins, IAA and NAA were proved to be less effective for root induction in these plant species as compared to IBA. The concentration of IBA and way of its treatment influences root induction (Van der Krieken et al., 1993). In *S. acidum*, it was found that 2, 4-D was outstanding to induce callusing than any other auxin (Gretchen, 2005) and experiments are underway to induce somatic embryogenesis in cell cultures as it is a good model system to study the molecular aspects of early plant development (Tchorbadjieva, 2006). The available germplasm should be propagated to fulfill increasing demands of society. Plant tissue culture technology can be used for germplasm conservation and mass propagation. Experiments are under progress to characterize (physiological, biochemical and molecular) rhizobial isolates for their plant promoting activities.

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