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Micropropagation of *Prosopis cineraria* (L.) Druce, An endangered tree of Pakistan

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Abstract

The present work was carried out to develop some newer techniques for the propagation of the endangered tree, *Prosopis cineraria*. For this reason, forcing of softwood shoots from large stem segments of *P. cineraria* was done. During dormant season, shoot were forced from large stem segments of jand. The large stem segments were placed in different growth media (Sand, peat moss and sawdust) under different environmental conditions (Dark and Natural). Results showed that peat moss logs produced the maximum number of shoots as compared to other tested media, sand and sawdust under both environmental conditions. Environmental conditions also had a significant effect as the dark condition gave promising results. Moreover, under natural condition, a fair number of shoots were produced in all media. But the sprouted logs soon turned dried due to harsh environmental conditions. While, the sprouted logs under dark conditions, on transferring into natural environment endured the harsh environment and established well. Therefore, the results suggests that this simple micropropagation technique developed for *P. cineraria* from stem cuttings can be exercised for large-scale rapid increase of this endangered tree.

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Introduction

Prosopis cineraria (L.) Druce, commonly known as jand belongs to the fabaceae. The plant is an evergreen, indigenous multipurpose wild tree growing in arid and dry areas of Pakistan, Afghanistan, India, Oman, Iran, United Arab Emirates and Arabia (Janbaz *et al.,* 2012; Muturi *et al.,* 2013). Jand is recognized as "Kalpatru" meaning "The king of desert" because of its food, medicinal and planted for reclamation and sand dune stabilization (Jordan and Balboa, 1985; Rani *et al.,* 2013). The pods are used as vegetable in green and dried form in many parts of the Thar Desert. The plant has some medicinal properties and used as a folk remedy for various ailments. The bark is refrigerant, anthelmintic and tonic used for dysentery, asthma, leucoderma, rheumatism, bronchitis, leprosy, piles, wandering of the mind and muscle tremors (Kirtikar and Basu, 1984; Malik and Kalidhar, 2007). The flowers mixed with sugar are used to prevent miscarriages (Orwa *et al.,* 2009). The monolayered canopy of the *Prosopis cineraria*, its capability of fix atmospheric nitrogen and deep root system, *P. cineraria* is to be compatible with agri-horticultural crops (Rani *et al.,* 2013; Pareek *et al.,* 2015). Furthermore, the promising multipurpose tree not only provides shade but also utilize as timber and domestic fuel wood (Kumar *et al.,* 2009). Its wood is used for boat frames, houses, posts, and tool handles (Orwa *et al.,* 2009).

Generally, *Prosopis cineraria* is propagated by seeds and established well with 80-90% germination (Mahoney, 1990). Propagation by air layering and root suckers has also been reported (Jordan and Balboa, 1985). But it seems that in vitro propagation is more challenging with *P. cineraria* than with other species (Pasiecznik *et al.,* 2004). In vitro expertise has not been exercised successfully in support of cloning mature and selected individuals of Prosopis tree because the explants taken from adult and aged plants do not rejoinder aptly in culture (Bovo *et al.*, 1991; Shekhawat *et al.*, 1993).

Prosopis cineraria is in the category of endangered species (Ali, 2015). Even though jand is a multipurpose tree but studies for its improvement are scanty all over the world. Survival of Prosopis cineraria is necessary for future generation (Pathak *et al.,* 2016). The present study aims to enhance the micropropagation technique such as, forcing large stem segments and secondly, to optimize conditions for the establishment of shoot segments for further vegetation or propagation.

Materials and methods

Plant material

A mature *Prosopis cineraria* (jand) tree growing near Pakistan Television Center, Multan was selected for the present research work.

Media preparation and sterilization

Three different types of growth media (Peat moss, sand and sawdust) were prepared to assess the micro-propagation and expansion of jand tree under natural as well as dark conditions. Sterilization is a pre-requisite for the tissue culture procedures and was done according to method prescribed by Misra and Misra (2012). The media (Peat moss, sand and sawdust) were sterilized by autoclaving at 15 lbs $inch^{-2}$ for 20 - 30 minutes at 121°C.

Forcing large stem segments

Stem segments of 40 cm in length (called as logs) varying in diameter were forced by placing horizontally in flats/ trays (52 × 25 × 6.5 cm; L × W × H) filled with sterilized peat moss, sand and sawdust. Three logs per flat were taken randomly and inserted 1/ 3rd into the respective medium according to layout prescribed by Aftab *et al.* (2005). These bags were kept under each testing condition for the rooting of the shoot segments. Standard light and temperature conditions were managed in the culture room under dark condition after sprouting to avoid chlorosis.

The cultures were placed under a 16 h photoperiod (35µmol m^{-2} $s^{-1)}$ provided by cool fluorescent tube lights at 25 ± 2°C.

Data collection and analysis

The data pertaining to sprouting of latent buds were recorded at the time of bud initiation. Analysis of variance (ANOVA) was made with SPSS release 21.0 for interpretation of results.

Results

In present study, a remarkable response was observed in terms of sprouting in all media under dark condition (Table-1). Sprouting was initiated in all growing media (sand, peat moss and sawdust) after 10 days. The maximum number of sprouting with greater number of shoot and length which having also maximum number of leaves and nodes was observed in peat moss under dark condition compared with sand and sawdust media (Table-1). The shoots were developed more rapidly in all media under dark condition specially grew well in peat moss. A cumulative influence of growing media under dark condition on shoot induction potential with special reference to all shoot derived parameters as shown in Fig.1. Under dark condition, the highest number of nodes and leaves were observed in peat moss followed by sand and sawdust respectively. Although, the shoots and leaves that were developed in dark conditions were yellow, after 30 days, on transferring under natural condition, shoots were turn into green (Fig.3). On the other hand, under natural condition, a fair number of sprouts were produced in all media. But these sprouts were survived for only 20 days and afterwards they turned out to be dried. A better response was observed in peat moss having maximum number of sprouts, shoots, shoot length, number of nodes and leaves when compared with sand and sawdust (Table 1, Fig. 3). The lowest response was observed in sawdust having reduced number of sprouts, shoots, shoot length, number of nodes and leaves (Fig. 2).

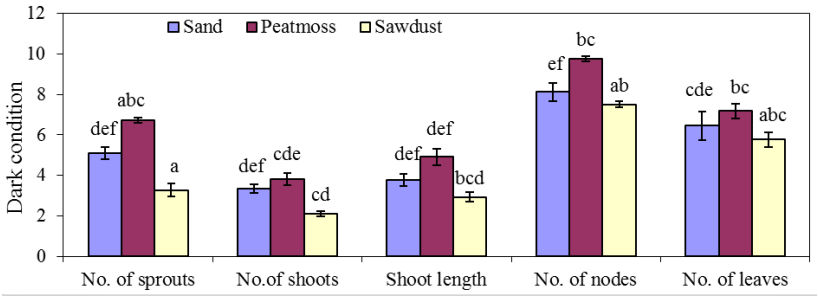

Fig. 1. Influence of different growing media (Peat moss, sand and sawdust) on sprouts induction potential of *Prosopis cineraria* under dark condition

Table-1. Effect of different media and environment conditions on forcing shoots from *Prosopis cineraria*.

Environmental Conditions	Medium (Sterilized)	No. of sprouts	No. of shoots	Shoot length (cm)	No. of nodes	No. of Leaves
Dark	Sand	5.09^{a}	3.34^{ab}	3.76^{abc}	8.12^{a}	6.54^{a}
	Peat moss	6.71^{a}	3.81^{abc}	4.91^{ab}	9.76^{a}	7.17^{a}
	Sawdust	3.26^{ab}	2.09^{cd}	2.93^{cd}	7.5^{a}	5.76^{a}
Natural	Sand	1.92^{def}	1.62^{abc}	2.24^{cd}	2.59^{cd}	2.2^{cd}
	Peat moss	2.52^{cd}	1.82^{def}	3.2^{abc}	3.65^{abc}	3.62^{abc}
	Sawdust	1.41^{def}	1.32^{def}	1.39^{def}	1.04^{def}	1.42^{def}

Note: The data presented here are the means of 12 logs per medium/environment and three logs were placed in each tray. Different letters here represents the significant difference at $P<0.05$ according to Duncan's Multiple Range Test.

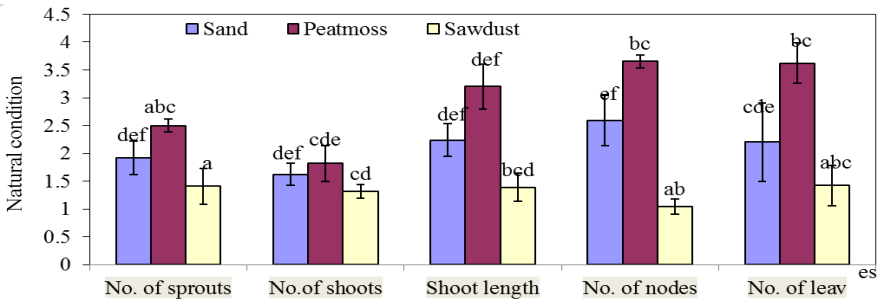

Fig. 2. Influence of different growing media (Peat moss, sand and sawdust) on sprouts induction potential of *Prosopis cineraria* under natural condition

Fig. 3. The stem cutting of *Prosopis cineraria* placed in different environments and media: (a) Stem cuttings placed in dark environment (b) Stem cuttings placed under natural environment (c) Sprouted stem logs in sterilized sand, peat moss, soil and sawdust media under dark environment (d) Sprouted stem logs in sterilized sand, peat moss, soil and sawdust media under natural environment.

Discussion

Many commercially important tree species can be propagated vegetatively by softwood stem cuttings (Henry and Preece, 1997). For clonal propagation and/or micropropagation, forcing softwood shoots from large stem segments of tree's juvenile portion is a progressive approach. The forced shoots could either be used as source of nodal explants for *in vitro* culturing process or for rooting process (Preece and Read, 2003). During the present research work, practice for mass propagation of the significant and endangered plant Prosopis cineraria, using the stem segments was developed (Fig. 3). Three media (sand, peat moss and sawdust) were tested for forcing large stem segments of *Prosopis cineraria*. Results showed that the maximum number of sprouting with higher number of shoot and maximum length which also having maximum number of leaves and nodes were observed in peat moss followed by sand media under

dark condition. While, the reduced number of sprouting, lesser number of leaves with nodes was observed in plant segments propagated in sawdust media. This ability to generate cutting material during the winter months has the potential to reduce the heavy spring workload associated with propagation tasks (Aftab *et al.,* 2005).

Similar technique was also employed for recalcitrant of woody species by Preece and Read (2003). Results also revealed that the stem segments under dark condition in all media; peat moss, sand and sawdust showed better outcomes when compared with forced large segments under natural condition. Though, the shoots and leaves that were developed under dark provision were yellow, but after 30 days, on transferring them under natural environment, shoots were turned into green. On the other hand, under natural condition, the highest numbers of softwood shoots were observed in sterilized peat moss media followed by sand and sawdust media.

Under natural condition, plants grew till 20 days after that sprouted shoots becomes necrotic and died finally due to sudden temperature change from winter to summer. Consequence of season on shoot propagation is as well accounted in woody plants such as *Prosopis cineraria* (Shekhawat *et al.*, 1993, *Maytenus emarginata* (Rathore *et al.*, 1992), *Terminalia bellirica* (Phulwaria *et al.*, 2012), *Delbergia sisso* (Arya *et al.*, 2013) and *Azadirachta indica* (Gehlot *et al.*, 2014).

Plants under natural conditions are also affected by various contaminants. Contamination is major problems that inhibit the softwood shoot production and growth. Arora *et al.* (2010) in *Azadirachta indica* and Phulwaria *et al.* (2011) in *Salvadora persica* also reported such kind of observations. Furthermore, the use of similar media and findings were reported by Akram and Aftab (2009) in the studies of teak plant. The peat moss can absorb water up to 20% of its weight, which is suitable to improve water holding capacity and nutrient contents at the initial stage which result in better establishment of stem logs.

Prosopis grows well in alluvial moderately saline soils where drainage is good and sufficient aeration (Kumar and Singh, 2009). Hence, Prosopis cineraria stem cuttings showed better sprouting and development in peat moss medium compared with sand and sawdust media under dark and natural environmental conditions.

Conclusion

An efficient and reproducible micropropagation practice has been devised using stem segments of mature *Prosopis cineraria*. Maximum sprouting response was observed in log placed under dark environment when compared with sprouting log placed under natural condition. The best in sprout induction result was obtained in stem cutting grown in peat moss medium under both conditions when compared with other media; sand and sawdust. Peat moss along with dark environment was found optimal for in sprouting and shoot establishment. The worth of this protocol is further enhanced as the sprouted logs endow with a regular furnish of shoots for field transfer devoid of any fresh explants.

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