International Journal of Agricultural Sciences
Volume **10** | Issue 2 | June, 2014 | 726-729
e ISSN–0976–5670 | Visit us | *www.researchjournal.co.in*

RESEARCH PAPER

In vitro approach for callus induction in kheemp [*Leptadenia pyrotechnica* (Forssk.) Decne] : A multipurpose plant

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Abstract : *Leptadenia pyrotechnica* (Forsk.) Decne belongs to the family Asclepiadaceae. It is commonly known as Kheemp in India. *L. pyrotechnica* is an important component of an arid ecosystem and source of fibre, forage, and medicines. In this present investigation, an efficient method has been developed for a rapid callus induction in *Leptadenia pyrotechnica*. After surface sterilization on MS medium supplemented with cytokinins and auxins (PGRs) individually and with various combinations, nodal, pod and inter-nodal explants from mature plant of *L. pyrotechnica* were cultured. Cultures were maintained at 30 ± 2°C temperature, 50–60 ìmol m^{-2} s^{-1} SFP, 16 hr day^{-1} photoperiod, and 62 per cent relative humidity (RH). Nodal segments proved the best explants (95% callus induction) compared with inter-nodal and pod explants had 11 per cent and 5 per cent callus induction, respectively. Different treatments were employed for surface sterilization of explants revealing that combination of sodium hypochlorite ($NaOCl_2$) and mercuric chloride (MC) were found significant. Minimum contamination (9%) occurred at 30 per cent $NaOCl_2$ + 2 g/L MC, while 85 per cent occurred at 30 per cent $NaOCl_2$ + 1 g/L MC. The nodal segments cultured on MS medium supplemented with 0.5 mg/L NAA that produced maximum callus (95%) within four weeks. It was followed by 65 per cent callus induction at 5 mg/L NAA + 2.5 mg/L benzyl-adenine (BA) and 25 per cent at 10 mg/L 2,4-D + 3 mg/L 2ip + 3 g/L activated charcoal (AC), while only 15 per cent appeared at control (without hormone application). This study may contribute in conservation management of this native plant species found especially in the Thar desert of Rajasthan.

Key Words : *Leptadenia pyrotechnica*, Arid environment, Cytokinins, Auxins, *In vitro*, Callus induction

View Point Article : Choudhary, Pratibha, Jhajharia, Arun and Singh, Dalel (2014). *In vitro* approach for callus induction in kheemp [*Leptadenia pyrotechnica* (Forssk.) Decne] : A multipurpose plant. *Internat. J. agric. Sci.*, **10** (2): 726-729.

Article History : Received : 09.12.2013**; Revised :** 27.04.2014**; Accepted :** 09.05.2014

INTRODUCTION

Leptadenia pyrotechnica (Forssk.) Decne is a desert herb belonging to family Asclepiadaceae is one of such medicinal plants, locally known as Khimp or Khip (Rajasthan), Broom bush (English) is an erect, ascending, shrub up to 1.2m-3.5m high with green stem and pale green alternating bushy branches with watery sap. Being highly drought-resistant, *Leptadenia pyrotechnica* has played an important role in the desert afforestation programs. The herb kheemp is a strong soil-binder and as such is one of the pioneer species in sand dune fixation due to prolonged and extensive root system. It is a valuable desert plant which is commonly used in traditional system of medicine for relieving pain and inflammation, as well as in a number of metabolic disorders such as diabetes and obesity (Rekha *et al.*, 2013). The plant is

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also used in thatching huts. The plant fibre is used for making ropes. The plant is browsed by all stock, but especially by camels for which it is considered a good fodder. Its flowers bloom in the late summer season. Its occurs in a leafless state almost throughout the year; only young shoots have leaves for a short period of time. The pods of this shrub known as "*khimpoli"*, ripe in the month of March which are of medicinal value and used as vegetables. The stems of *Leptadenia pyrotechnica* can be harvested whenever the need arises. It is common throughout the state of Rajasthan and found in dry habitats particularly in desert zones (Shetty and Singh, 1991). Whole plant seeds and flowers are used for different purpose as its fibre is used as antihistaminic and expectorant (Al-Yahiya, 1986). Fresh juice of the plant is used for abortion (Patel *et al.*, 2010). Plant sap is applied to eczema and other skin disease and is also given in diabetes (Kateva and Galav, 2006). Whole plant is used proved to have antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Al-Fatimi *et al.*, 2007; Praveen *et al.*, 2007). The latex or the leaf paste is applied over the thorn injury for thorn removal (Upadhyay *et al.*, 2010). Medicinally, the whole plant is boiled in water and given to cattle after delivery for the expulsion of placenta (Bhatti *et al.*, 2001). Whole plant infusion is mixed with buttermilk and given for uterine prolapse and stomach disorders in sariska region of Rajasthan (Upadhyay *et al.*, 2011). It is used to cure constipation and is considered good for health in Bikaner region of Rajasthan (Dagla *et al.*, 2012). *Leptadenia pyrotechnica* has furthermore potential to be developed as a fibre plant for industrial use, and also has much potential as a stabilizer of sandy soils.

In vitro culture of plants has gained importance during recent years because this technique can be used for the rapid multiplication and *ex situ* conservation of some plants. There are very few reports (Dagla *et al.*, 2012; Qureshi *et al.*, 2012). are available about micro-propagation of *L. pyrotechnica* and the aim of the present work was to determine the culture conditions for micro-propagation of this plant.

MATERIAL AND METHODS

This research work was undertaken in the Department of Plant Breeding and Genetics and Biotechnology Centre, College of Agriculture, Rajasthan Agricultural University, Bikaner. The plant materials were collected from different plants from various regions of Thar Desert of Western Rajasthan. Young stem nodal segments, pods and inter-nodal segments were selected as an explant source. One explant (shoot tip) was inoculated on a flask and each treatment was replicated 10 times and data were analyzed by using Complete Randomized Design.

Media preparation :

Throughout the course of investigation, Murashige and Skoog (1962) medium was used. Readymade vial of MS media (Hi-Media laboratories, Mumbai) were mixed with supplements and final volume was made after dissolving the sugar, agar and double distilled water. The 1000-ppm stock solution of 2 different plant growth regulators (PGRs) auxin (IAA, IBA and NAA) and cytokinins (KN and BAP) were prepared for the induction of callus. For preparation of stock solution 100 mg of PGR was dissolved in 100 ml of distilled water. Auxins were initially dissolved in a few drops of alcohol and cytokinins were dissolved in a few drops of 1 N NaOH and final volume was made up by adding distilled water. The Nutrient medium either alone or supplemented with different combinations of PGRs (Table 1) was used with MS as a basal medium containing (in mg L^{-1}): 100.0 myo-inositol, 1.0 nicotinic acid, 1.0 pyridoxine-HCl, 1.0 thiamine-HCl, 2.0 glycine, 200.0 glutamine, 40.0 adenine sulfate, 2400.0 agar, 1400.0 Gel (Hi-Media laboratories, Mumbai) and 30000.0 sucrose. After preparation of the medium, pH was adjusted to 5.8 ± 0.2 before autoclaving. Media were dispensed into small culture tubes (30 × 150 mm) in aliquots of 20 ml per tube and were capped with aluminum foil. The same were then autoclaved for 20 min at 1.11 kg/cm^2 and 121^{0}C and stored at 4^{0}C before inoculation with explants (Qureshi *et al.*, 2012).

Explant surface sterilization :

Freshly harvested nodal, pods and internodal segments were cleaned and dead/decaying parts separated, that were washed with the running tap water for 15 min to remove the dust or sand particles. These were then surface sterilized by using two different methods. In the first method, all explants were treated with 75 per cent ethanol for 5 min and then washed with 50 per cent sodium hypochlorite (bleach) for 20 min and then dispensed thrice with autoclaved distilled water to remove bleach completely. In the second method, explants were treated with 30 per cent of sodium hypochlorite for 15 min and then immersed in 1 and 2 g/L MC solution for 3 min. After that, the plant material was washed three times with sterile distilled water with gentle shaking under sterile conditions (Qureshi *et al.*, 2012).

Inoculation of explants :

On both portions (basal as well as the top) of the explants, a cut was given to remove undesirable/dead portions after surface sterilization. All explants were then transferred in the test tube containing medium while keeping them in an erect position. All of the explant cultures were maintained in controlled environment of growth room under illumination, provided by white fluorescent tube lights, with a photoperiod of 16 hour per day (Qureshi *et al.*, 2012). Cultures were visited regularly to observe their response to tissue culture. Data were recorded after every week, for four weeks and the values presented as scores as +, ++, +++, - represent poor, moderate, high and no response, respectively, according to the method described by Abul-Soad *et al.* (2002) and Mujib *et al.* (2005).

Table 1 : Effect of different medium compositions on callus formation

Conc. No.	Media composition (mg/L)	Replicates/ treatments	Percentage age of callus induction nodal explants	Percentage age of callus induction pod explants	Percentage age of callus induction internodal explants
1.	MS	10	15	-	-
2.	MS + 0.5 NAA	10	95*	-	-
3.	MS + 1 IAA	10	-	-	-
4.	MS + 0.1 IAA + 1 BAP	10	-	-	-
5.	MS + 0.1 NAA + 1 BAP	10	-	-	-
6.	MS + 0.5 BA	10	20	-	-
7.	MS + 0.5 NAA + 0.5 BA	10	30***	-	-
8.	MS + 0.5 NAA + 2.5 BA	10	65**	-	-
9.	MS + 2.5 NAA + 0.5 BA	10	20	-	-
10.	MS + 10 2,4-D + 3 2ip + 3000 A.C	10	25***	-	-

*, ** and ** indicate significance of values at P=0.05, 0.01 and 0.10, respectively

Callus induction rate on each media formulation was calculated using the following equation:

$$\text{Percent response} = \frac{\text{Number of explants responded}}{\text{Total number of explant inoculated}} \times 100$$

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Effect of sterilization method :

The surface sterilization was optimized that helped in preventing blackening of tissues and establishment of clean cultures. The sterilization with 30 per cent $NaOCl_2$ + 2 g/L MC proved the most successful procedure as all of the cuttings were undamaged and responded to tissue culture medium which sprouted well and only 9 per cent of them became infected. On the other hand, treatment with 70 per cent ethanol + 50 per cent $NaOCl_2$ and 30 per cent $NaOCl_2$ + 1 g/L MC failed due to contamination of all explants.

Effect of plant material :

For callus induction, nodal segments found the best source of explants that showed 95 per cent response as compared with inter-nodal and pod explants. Except nodal explants, rest of the materials expressed 11 per cent with very poor or nominal 3 per cent response, respectively in callus induction on any media formulation (Table 1) due to vigor loss which became gradually dead, similar results were also reported by Qureshi *et al.* (2012).

Callus induction :

Auxins and cytokinins are major growth regulators that have profound influence on various phenomena of cell division, callus induction and regeneration Munazir *et al.* (2010), Tang *et al.* (2000). In the present study, maximum callus induction (95%) was observed when 0.5 mg/L of NAA was used, followed by 2.5 mg/L of BA (65% callus induction) and 0.5 mg/L of NAA + 0.5 mg/L BA and 10 mg/L of 2,4-D + 3 mg/L 2ip + 3000 AC which showed 25 per cent callus induction, respectively (Table 1). The rest of combinations of NAA + BA, NAA + BAP, IAA + BAP and 2, 4-D + 2ip + A. C were capable of producing more or less poor results. The control treatment (that is, MS medium without any hormone) was capable to induce callus only in a trace amount. Rapid callus induction and its proliferation are vital to tissue culture as those calli are of no use which fails to proliferate (Qureshi *et al.*, 2012); Munazir *et al.*, 2010). It was observed that when MS was supplemented with 0.5 mg/L of NAA, it did not only result in maximum callus induction but also calli proliferated well at this concentration. This shows that a suitable concentration of growth regulating substance is fruitful in tissue culture for further propagation (Qureshi *et al.*, 2012).

Conclusion:

The present study proposes nodal segment as an effective explant which expressed maximum callus at 0.5 mg/L NAA.

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