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CALLUS INDUCTION FROM THE LEAVES OF ELEPHANT BUSH *PORTULACARIA AFRA JACQ.* ON MODIFIED MS MEDIUM

Biological Science

Dr. Madhusudan S* Department of Biotechnology, UG and Research Centre, Maharani's Science College for Women, Palace Road, Bangalore – 560001 *Corresponding Author

Veena M.R Department of Biotechnology, UG and Research Centre, Maharani's Science College for Women, Palace Road, Bangalore – 560001

Dr. Keshamma E Department of Biochemistry, PG and Research Centre, Maharani's Science College for Women, Palace Road, Bangalore – 560001

ABSTRACT

Portulacaria afra Jacq., commonly Called as porkbush or elephant bush is a popular succulent garden plant in use around the world and is often used for bonsai. It has now been shown to be effective in carbon sequestration (binding atmospheric carbon which is responsible for climate change), in semi-arid landscapes and thicket vegetation. It is also being used for restoration purposes. In view of the importance of this plant, MS medium was optimized for callus induction from the leaves of the *P. afra*. The leaves were used as the explants for callus induction on modified MS media at different hormonal concentrations. The optimum medium for callus induction from the leaves of *P. afra* was found to be MS+1.5 mg/L 2, 4-D and 0.5 mg/L of BAP with slight modification of micro nutrients and vitamins under optimal culture conditions. The callus was well induced on the modified MS medium.

KEYWORDS

P.afra, 2,4-D, BAP, callus induction

Introduction

Portulacaria afra or porkbush (Fig. 1) is a popular succulent garden plant in use around the world and is often used for bonsai (Arnold and De-Wet, 1990). It has now been shown to be effective in carbon sequestration (binding atmospheric carbon which is responsible for climate change), in semi-arid landscapes and thicket vegetation. It is also being used for restoration purposes (Arnold and De-Wet, 1990).

The porkbush is an attractive, evergreen succulent shrub or small tree that can reach 2 - 5 m in height. The flowers of the plant are rich source of nectar for many insects, which in-turn attracts insectivorous birds (Sim, 1907; Jackson, 1990; Joffe, 1993). *P. afra* is listed in the National Red Data List as Least Concern which indicates that it is not threatened in its natural habitat (Palmer and Pitman, 1961; Raimondo, 1990). It is found in warm situations on rocky slopes in succulent karoo scrub, thicket, bushveld and dry river valleys in the eastern parts of South Africa (Jackson, 1990; Bruyns et al., 2014). The name *Portulacaria* is composed of Portulaca + aria suggesting a similarity to the genus Portulaca. The word afra is in reference to the fact that the plant occurs in Africa. The porkbush belongs to a large and widespread family (Portulacaceae) (Jackson, 1990; Bruyns et al, 2014).

Interesting ecology has been observed with this plant in the Eastern Cape where it forms part of the diet of the Addo elephants. A very interesting mistletoe, Viscum crassulae, parasitizes this plant. The porkbush has also been indicated as a soil binder for preventing soil erosion (Oliver, 2009; Pooley, 1993). Traditional uses also include the increasing of breast milk by lactating mothers. The leaves are used to quench thirst, sucking a leaf is used to treat exhaustion, dehydration and heat stroke. Crushed leaves can be rubbed on blisters and corns on the feet to provide relief. The leaves are chewed as a treatment for sore throat and mouth infections while the astringent juice is used for soothing ailments of the skin such as pimples, rashes and insect stings. The juice is also used as an antiseptic and as a treatment for sunburn. (Roberts, 1990; Oliver, 2009; Pooley, 1993; Van-Wyk et al., 1997).

Recent research has shown the Porkbush to be an excellent 'carbon sponge' as it has the ability to sequestrate (absorb) free carbon from the atmosphere which is used to make plant tissue (Mills and Cowling, 2006; Stern, 1996). Carbon is one of the major greenhouse gases which are responsible for the warming of the earth's atmosphere; it is produced in excess by burning of fossil fuels. Currently, humans are producing atmospheric carbon faster than the environment can absorb it, causing a deficit which remains in the atmosphere and causes heat from the sun to be trapped instead of being radiated back out into space. The porkbush has the unique ability to absorb more carbon from the atmosphere than most other plants and consequently has the ability to remove more carbon from the atmosphere than an equal amount of deciduous forest (Mills and Cowling, 2006; Stern, 1996). Reports indicate that the plant is rarely propagated by seeds but rather by cuttings (Palmer and Pitman, 1961; Bruyns et al., 2014). Till date the valuable plant *P. afra* have not been propagated by tissue culture techniques and hence in view of the importance of this plant, MS medium was optimized to induce callus from the leaves of the *P. afra* with different concentrations of auxins and cytokines and slight modification in micronutrients and vitamins.

Fig. 1: *Portulacaria afra* or porkbush

Materials and Methods

Plant Material

The potted plants of *P.afra* were obtained from Lalbagh Botanical Gardens, Bengaluru, Karnataka and brought to the laboratory. The plants were maintained in the laboratory premises by potting the cutting's of the plant in fresh pots containing fertile soil under natural conditions. The leaves were collected from the one month old healthy plants for callus induction.

Preparation of Culture Medium

The basal medium used was of Murashige and Skoog (1962) (MS) salt solutions without hormones. Callus induction from the leaves of *P. afra* was obtained by using MS medium modified with 2,4-Dichlorophenoxyacetic acid (auxin) and 6-Benzylaminopurine (cytokinin). The callus was induced on the media contained MS salts, vitamins, 30g/L sucrose, 8g/L agar agar (Hi-Media), 2,4-Dichlorophenoxyacetic acid (Hi-Media) at concentrations of 0.5, 1.0, and 1.5 mg/L, 6-Benzylaminopurine (Hi-Media) at concentrations of 0.1, 0.25 and 0.5 mg/L. The pH was adjusted during each experiment to 5.8 with 1N NaOH and 1N HCl with electronic pH indicator. All the operations were carried out under aseptic conditions and the medium was heated to dissolve solutes and dispensed in culture bottles and autoclaved at the temperature of 121° C at a pressure of 15lbs psi for 20 minutes and cooled at room temperature (Puchooa et al., 1999).

Surface sterilization of the explants

Leaves of *P. afra* were taken as explants for callus induction. Surface sterilization of the explants was performed according to (Niedz and Bausher, 2002) with modifications. The explants were collected from the plant, washed in running tap water to remove the dust and other contaminants present on the surface of the explants. Then rinsed with distilled water for 1minute and this step was repeated three times. The explants were then immersed in a mild detergent for about 15 minutes and again washed with double distilled water for 1 min thrice. The explants were then taken into laminar hood for further surface sterilization steps. The explants were transferred into a sterile conical flask containing ethanol (70%) and rinsed for 30 seconds. This step was followed three times followed by rinsing with double distilled water for 1 min three times. This step was followed by treating the explants with mercuric chloride (0.1%) (Hi-Media) for 3 minutes followed by rinsing with double distilled water. The explants were then blot dried using a sterile blotting paper and transferred to sterile culture bottles containing modified M S medium for callus induction.

Callus induction on modified MS medium

For callus induction, different media compositions were used individually. All the compositions were prepared using basal MS media (Murashige and Skoog, 1962), supplemented with different concentrations of 2,4-Dichlorophenoxyacetic acid (Hi-Media) at 0.5, 1.0, and 1.5 mg/L and 6-Benzylaminopurine (Hi-Media) at 0.1, 0.25 and 0.5 mg/L (Table.1) with 30 gm/l of sucrose. Agar (8%) was used as solidifying agent for all the composition. The surface sterilized explants were transferred aseptically to all the media compositions and labelled accordingly. All the culture bottles were kept under 16 hrs photo period provided by white fluorescent tube lights (1000 lux) at 25±1 °C for four weeks.

Table:1. Concentrations of PGR used for Callus Induction

Explant Type	Medium Type	PGR Conc. In mg/L	
		2,4-D	BAP
Leaves	MS-0	0.0	0.0
	MS-1	0.5	0.1
	MS-2	1.0	0.25
	MS-3	1.5	0.5

Results

Callus induction from the leaves of *P. afra* on modified MS medium.

In the present study we have used leaf segments for callus induction on modified MS medium containing different concentrations of 2,4-D and BAP. The highest percent of callus formation was observed in MS medium with 1.5 mg/L 2,4-D and 0.5 mg/L BAP hormonal concentration (Plate-3), medium percent of callus formation was observed in MS medium with 1.0 mg/L 2,4-D and 0.25 mg/L BAP hormonal concentration (Plate-2) and least percent of callus formation was observed in MS medium with 0.5 mg/L 2,4-D and 0.1 mg/L BAP hormonal concentration (Plate-1). Hundred percent of callus induction was seen on explants inoculated on MS-3 medium and the degree of callus induction was massive on this medium, similarly 60 percent of callus induction was seen from the explants inoculated on MS-2 medium with moderate degree of callus induction and 40 percent of callus induction was seen from the explants inoculated on MS-1 medium with slight degree of callus induction and no callus induction was seen from the explants inoculated on MS-0 medium without any hormones, was used as the control in the current studies (Table. 2). Finally the effective and massive callus induction was found to be on modified MS medium with 1.2 mg/L 2,4-D and 0.5 mg/L BAP hormonal concentration,

Table:2. Effect of PGR on Callus Induction

Explant Type	Medium Type	PGR Conc. In mg/L		% of Explants Induced Callus	Degree of Callus formation
		2,4-D	BAP		
Leaves	MS-0	0.0	0.0	0	-
	MS-1	0.5	0.1	40	+
	MS-2	1.0	0.25	60	++
	MS-3	1.5	0.5	100	+++

---=no callus, +=slight callus, ++=moderate callus, +++=massive callus

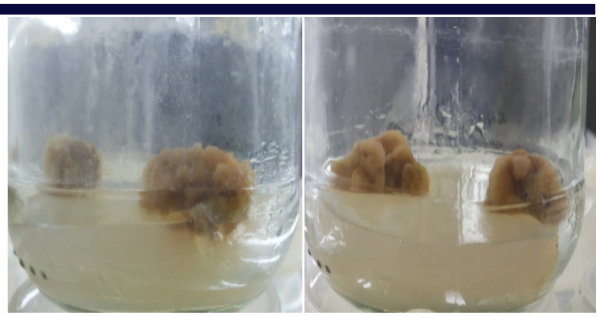

Plate-1: Callus induction from leaves of *P. afra* on MS medium with hormonal concentration of 0.5 mg/L 2,4-D and 0.1 mg/L BAP

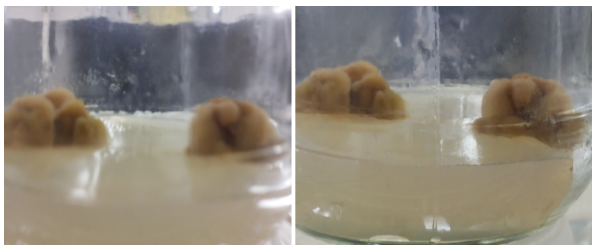

Plate-2: Callus induction from leaves of *P. afra* on MS medium with hormonal concentration of 1.0 mg/L 2,4-D and 0.25 mg/L BAP

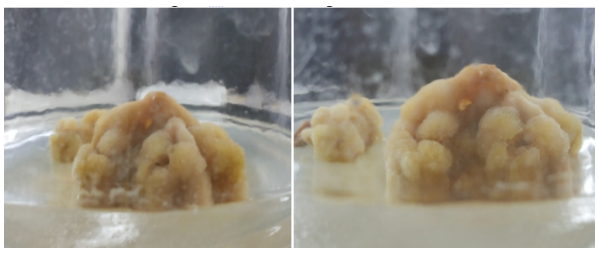

Plate-3: Callus induction from leaves of *P. afra* on MS medium with hormonal concentration of 1.5 mg/L 2,4-D and 0.5 mg/L BAP.

Discussion

The laboratory experiments were conducted to optimize the conditions for callus induction from the leaves of *P. afra*. The important criteria for plant tissue culture is to avoid contamination and establish aseptic culture using explants which provide high risk of internal and external contamination (Misaghi and Donndelinger, 1990). Surface sterilization of the explants (Niedz and Bausher, 2002) was performed to obtain contamination free culture of the various media formulations. The experiments were conducted under aseptic conditions and no contamination was observed in each culture, no rot and mold were observed around the leaf inoculants and aseptic conditions were maintained throughout the experiments. Callus were induced in four weeks by taking leaf explants from in vitro grown plants of *P. afra* on modified MS media supplemented with various concentrations of cytokinins and auxins. Callus was observed after four weeks of the culture in all the combinations tested. The effective and massive callus induction was found to be on modified MS medium with 1.5 mg/L 2,4-D and 0.5 mg/L BAP hormonal concentration. Callus induction from leaf explants on MS medium with various combinations of cytokinins and auxins in different other plant species have been reported (Clemente, 2006; Geethalakshmi et al, 2016). There is no studies have been carried out till date on tissue culture techniques for this model plant. This plant has been gaining importance recently as an excellent 'carbon sponge' as it has the ability to sequestrate (absorb) free carbon from the atmosphere which is used to make plant tissue (Mills and Cowling, 2006; Stern, 1996). The porkbush has the ability to make use of two different photosynthetic pathways, when conditions are favourable it manufactures its food to sustain growth by using the same method (pathway) that most other plants use. However, when conditions are not favourable and other plants have to shut down and wait for sufficient rain, the porkbush can switch to a different pathway called CAM (Crassulacean Acid Metabolism) whereby it can continue to grow and slurp up huge amounts of carbon despite adverse climatic conditions. This allows the plant to excel in the arid or semi-arid conditions (Mills and Cowling, 2006; Stern, 1996). Thus, this study

describes effective procedure for *in vitro* culture and propagation of *P. afra* in order to use as CO_2 absorber in CO_2 polluted environments.

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