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MICRO PROPAGATION OF *CARISSA MACROCARPA* L. PLANT *IN VITRO*

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Abstract

Carissa macrocarpa L. is an important medicinal plant are used for various medical treatments. An efficient micro propagation protocol was developed for using nodal segment as the explants. After sterilization of explants, no contaminations were recorded. Explants were cultured on MS medium supplemented with various concentrations of BA and 0.5 mg/l NAA. The higher average of shoots Number 4.30 per explant and mean length of shoots 1.25 cm and maximum fresh and dry weight of shoots induction 442.0, 38.4 mg respectively in nutrient medium supplemented with 1mg/l of BA+0.5 mg/l of NAA. Results of multiplication stage showed that the best multiplication was on the medium supplemented with 3mg/l of BA+0.5 mg/l of GA_3, induced large number of shoots reached 5.70 and the highest length of multiple shoots 1.64 cm with maximum fresh and dry weight of shoots 539.7, 51.20 mg, respectively. Rooting was readily achieved upon transferring the shoots on to half-strength MS medium the highest average number of roots was 2.80 while the highest average of root length was 1.50 cm at the concentration of 4.5 mg/l of NAA. Rooted plants were successfully acclimatized to greenhouse conditions.

Key words : Micro propagation, Apocynaceae, *Carissa macrocarpa*.

Introduction

Carissa macrocarpa L. belonging to the family Apocynaceae Carissa is a genus of the family Apocynaceae, with about 36 species as evergreen shrubs native to tropical and subtropical regions of Africa, Asia and Oceania (Kaunda and Zhang, 2017). They are cultivated as ornamental plants in America ranging from Florida to California. Common species belonging to this genus are *C. macrocarpa*, *Carissa carandas*, *C. grandiflora*, *C. edulis*, *C. spinarum*, *C. lanceolata*, *C. opaca*, *C. congesta* and *C. bispinosa* (Nedi *et al*., 2004). *Carissa macrocarpa* is a shrub native to South Africa. It is commonly known as the Natal plum and in South Africa, the large num-num, in Zulu (Wiart, 2006). Like other *Carissa* species, *Carissa macrocarpa* is a spiny, evergreen shrub with short stem and strong thorns in pairs (Malik *et al*., 2010). The rich green leaves are glossy, waxy and tough oval-shaped while the white flowers are fragrant. The shrubs are very attractive as hedge plants for the dense branches and star-like flowers (Nedi *et al*., 2004). They bloom for months at a time. The ornamental plump round fruit appears in summer and fall (autumn) at the same time as the blooms (Wiart, 2006). The fruit can be eaten and contained Ca, Mg, Fe, Mn, Cu, Pb, Se, Ni and Zn in decreasing order. The Pb content is low and permissible by dietary recommendations. Lipid profiling revealed the appreciable monounsaturated and essential fatty acid contents (Moodley *et al*., 2012) in most of Carissa plants has been used as a traditional medicinal plant over thousands of years in the high blood pressure, antihypertensive, anti-inflammatory, antiplasmodial, decrease blood glucose level (AL-Youssef and Hassan, 2014). The rural poor for the treatment of HIV related infections such as venereal diseases, tuberculosis, diarrhea and appetite loss have also been reported by Bodeker *et al*. (2002) and King (2002) as well as anti-cancer, anti-oxidant, analgesic, anti-ulcer, anthelmintic activity, cardiovascular, anti-nociceptive and DNA damage inhibition (Singh and Uppal, 2015). Propagation of *Carissa* is done via seeds, cuttings, grafting, air layering and stooling. However, these methods are season specific and take long time to propagate. Whereas, micro propagation is a tissue culture method for producing thousands of plantlets in a short span of

time, through culturing explants in controlled laboratory environmental condition.

Seeds of *Carissa* have short viability and should be sown just after extraction from fruits (Kumar *et al*., 2007). Due to low percentage of seed viability and destructive collection of roots from trees, limits its natural propagation. Hence, alternative methods like *in vitro* techniques could be used to propagate this plant and there by multiply elite genotypes.

Materials and Methods

Surface sterilization of explants

Explants of nodal segment was excised from shoot cuttings of *Carissa macrocarpa* L. and sterilized by washing in tap water running for 60 minutes to remove all adhering dust and phenols and immersed in 4.5% of NaOCl (6% sodium hypochlorite) for 12 minute and small drops of Tween 20. Then washed three times with sterile distilled water in laminar air flow hood to remove the residuals.

Culture media and conditions

All MS media were supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} Agar-agar. The pH of all media was adjusted to 5.7 with 1 N NaOH or 1 N HCl prior to auto-claving at 121ºC, 1.5 kg/cm^3 for 15 min. Cultures were maintained at 25±1^{0}C air temperatures in a culture room with a 16/8 h light/dark and each screw glass tubes (85 × 28 mm) containing 10 ml of medium.

Shoot induction

Sterilized nodal explants were used for shoot initiation in media full strength of Murashige and Skoog (1962) (MS) medium supplemented with benzyl adenine (BA) at 0.25, 0.5 and 1.0 mg/l combination with 0.5 mg/l NAA. MS basal medium without growth regulators served as the control.

Multiplication of shoot

Shoot multiplication was induced with the help of (MS) medium supplemented with benzyl adenine (BA) at 1.0, 2.0, 3.0, and 4.0 mg/l combined with 0.5 mg/l gibberellic acid (GA_3).

Induction of root

Elongated shoots with an average height of 1 cm were excised from culture tubes and transferred to half strength of MS medium supplemented with 0.0, 1.5, 3.0 and 4.5 mg/l NAA to induce roots.

Acclimatization of plantlets

After rooting, plants with well-developed roots were removed from culture tubes, washed gently with running tap water, plantlets were transferred to corks cups containing combinations (1:1) of sand and peat a sterile. These plantlets were covered with glasses for maintaining high humidity and were maintained inside the culture room conditions. The relative humidity was reduced gradually, and then the plantlets were kept in partial shade for acclimatization (2-3 weeks). Finally, the plantlets were exposed to the normal conditions and transferred to the field.

Statistical analysis

The experiment was planned under completely randomized design with different treatments with ten replications. The data thus generated were analyzed through one way analysis of variance (ANOVA) and the treatments means were compared for significance by L.S.D, using Genestat.

Results and Discussion

Shoot induction from nodal explants

The results showed in table 1, that the nodal segment cultured on MS medium fortified with various concentrations of BA combination with 0.5 mg/l NAA. The best results were obtained with 1mg/L of BAP+0.5 mg/l of NAA, highest average of shoots, number 4.30 per explant and mean length of shoots 1.25 cm and maximum fresh and dry weight of shoots induction 442.0, 38.4 mg respectively were significantly higher than the media containing 0.25 mg/l of BAP+0.5 mg/ l of NAA.

Our results were supported by Ananthi *et al*. (2011), who reported that some species may require a low concentration of auxin in combination with high levels of cytokines to increase shoot proliferation and auxins are taken up by the cells through pH trapping or by influx carrier proteins. Auxin signal is perceived right in the heart of nucleus. Auxins control the transcription of some genes in the nucleus which in turn control the cell wall elongation. From the results, it is evident that higher concentration of cytokinin in combination with Auxin could induce multiple shoot formation and multiple shoot elongation from nodal segments are agreed results with findings of Salma *et al*. (2008), Bhatt *et al*. (2008) and Susila *et al*. (2013) on *Rauwolfia serpentina* L. A similar induced shoot formations from the nodal segments of *Carissa carandas* has been reported by Hasmah *et al*. (2013).

Induction of multiple shoots

Data represented in table 2 that the concentration of BA 3mg/l + 0.5 mg/l of gibberellic acid (GA_3), which differed significantly from the other treatment was the best to give the highest number of multiple shoots reached

Fig. 1 : Shoot proliferation with MS medium was at 3.0 BA and 0.5 GA_3.

multiple shoots 1.60 per explant and length for multiple shoots 1.28 cm with less fresh and dry weight of shoots 80.5, 7.40 mg, respectively. BA is used in the multiple shoots of many plant species as a source of cytokinin. This is due to its effectiveness in releasing the axillary buds from the dominance of the terminal bud without the need for remove it. It is believed that cytokinin stimulates the formation of wood tissue for the buds and leg, making it easier water and nutrients flow and the lateral bud grows and the importance of addition of cytokinin lies in stimulating cell division and stimulating the formation and growth of transverse and alternator branches (Zibbu and Batra, 2010).

The use of high concentrations of BA produced short

Fig. 2 : Rooting response from NAA at 4.5 mg/l.

Fig. 3 : Acclimatized plantlet.

5.70 shoots per explant and mean length of multiple shoots 1.64 cm with maximum fresh and dry weight of shoots 539.7, 51.20 mg respectively (fig. 1). Compared to the media containing 4mg/l of BA+0.5 mg/l of GA_3, which recorded the lowest rate of the average number of shoots and this is achieved by both Anushi and Jahan (2009) on *Dodonea viscose*. So the presence of GA_3 induced shoot elongation and these are agreed results with finding Gonbad *et al*. (2014) on *Camellia sinensis* was maximized at the concentration of BA 3 mg/l in combination with 0.5 mg/l GA_3 had multiple shoots with a height length. And a similar micro propagation protocol for *Ficus carica* from nodal explants has been reported by Darwesh *et al*. (2014) GA_3 combined with BA at 3 mg/l compared with the control.

Rooting and acclimatization

Data of shoot grown on MS medium including different concentrations of NAA in table 3 showed that the high concentration of NAA at 4.5 mg/l (fig. 2) induced high frequency number of roots, which gave 2.80 was significant compared with the treatment of NAA at 3.0 mg/l, which gave 1.10 number of roots per shoots. While average length of longest root (1.50 cm) on 4.5 mg/l NAA was not significant with shortest length of root (1.46cm)

Table 1 : Effect of different concentrations of BA combination with NAA into the MS medium on Induction shoots from nodal of *C. macrocarpa* L after 4 weeks of culture.

Dry weight (mg)	Fresh weight (mg)	Length of shoots (cm)	Number of shoots	Treatment BA+NAA (mg/l)
0.0	0.0	0.0	0.0	0.0 +0.0
5.3	59.0	0.30	0.70	0.25+0.5
11.5	115.0	0.65	2.50	0.5 + 0.5
38.4	442.0	1.25	4.30	1.0 + 0.5
13.83*	155.8*	0.58*	1.89*	L.S.D 0.05

* In each column means significantly different.

Table 2 : Effect of different concentrations of BA combination with GA_3 into the MS medium on multiple shoots of *C. macrocarpa* L after 4 weeks of culture.

Dry weight (mg)	Fresh weight (mg)	Length of shoots (cm)	Number of shoots	Treatment (mg/l) BA+ GA_3
0.0	0.0	0.0	0.0	0.0 +0.0
11.90	137.0	1.49	2.20	1.0+0.5
28.40	320.5	2.56	3.60	2.0+0.5
51.20	539.7	1.64	5.70	3.0+0.5
7.40	80.5	1.28	1.60	4.0+0.5
3.07*	30.1*	0.28*	0.48*	L.S.D 0.05

* In each column means significantly different.

Table 3 : Effect of NAA supplemented in to the MS medium on rooting of *Carissa macrocarpa* L. after 5 weeks of culture.

Length of roots (cm)	Number of roots	Treatment NAA (mg/l)
0.00	0.00	0.0
0.00	0.00	1.5
1.46	1.10	3.0
1.50	2.80	4.5
0.34	0.53*	L.S.D 0.05

* In each column means significantly different.

on 3.0 mg/l NAA. No rooting was observed at low concentration of NAA as well as control treatment. Shoots developed were rooted best with NAA 10.74 µM. Optimum root multiplication was obtained by Sri Rama *et al*. (2010) on *Ceropegia spiralis* L. Supporting results were obtained by Ramachandra *et al*. (2017), who reported that the NAA showed positive response of rooting when compared with IAA and IBA in *Caralluma adscendens*.

The *in vitro* rooted micro shoots survived and grew well in the pot containing sterile sand and garden soil for hardening and acclimatization and initial hardening under culture room condition acclimatization (fig. 3). These established plants exhibited 90% survival under field conditions.

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