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MICROPROPAGATION OF *JARTORPHA CURCAS* L. THROUGH SHOOT TIP EXPLANTS USING DIFFERENT CONCENTRATIONS OF PHYTOHORMONES

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ABSTRACT

A study was conducted to evaluate the effect of phytohormone BAP alone and in combination with IBA or NAA for in-vitro propagation of *Jatropha curcas* shoot tip explants. MS medium supplemented with various concentrations of phytohormones significantly affected shoot induction. Maximum (93.33%) shoot induction occurred when MS medium was supplemented with 8.88 µM BAP or 13.32 and 4.92 µM of BAP and IBA respectively. Similarly, maximum shoot buds per explant (6.7) and shoot length (4.5 cm) were obtained when 13.32 µM BAP and 4.92µM IBA was added to the MS medium. Shoots produced were re-cultured to MS, ½ MS media and ½ MS medium supplemented with different concentrations of IBA and were studied for rooting, where the response was highly significant. IBA supplemented to ½ MS medium at 14.76µM gave maximum (4.93) roots per culture.

Key words: *Jatropha curcas*, micropropagation, phytohormones, BAP, IBA.

INTRODUCTION

*Jatropha curcas L. (*physic nut) belonging to the family *Euphorbiaceae* is a multipurpose plant. Seed kernel contains 33-40 % oil especially used for its medicinal properties and biodiesel production (Datta *et al*. 2007, Heller 1996, Staubmann 1999, Openshaw 2000). The oil is known as curcas oil having high value and can be used directly in diesel engines added to fuel as an extender. Pure Jatropha diesel is non-toxic in nature. The oil has greater lubricity and reduces engine wear. The oil is strong purgative, widely used as an antiseptic for cough, skin diseases and as pain reliever, produces glycerin as by-product, which is in great demand for cosmetic, medicine and food product industries (Datta 2007). The leaves are used in different forms in West Africa in ethno-medical practice to cure fever, mouth infections, jaundice and guinea worm sores (Irvine, 1961; Oliver-Bever, 1986).

The water extract of the branches strongly inhibited the HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*. 1999). The roots are incorporated in products used to cure bleeding gums, toothache, ringworm, scabies, eczema, dysentery and venereal diseases such as gonorrhea. It is also reported that the root methanol extract exhibit anti-diarrhea activity in mice through reduction of osmotic pressure and inhibition of prostaglandin biosynthesis (Oliver-Bever 1986; Mujumdar *et al*. 2001). The active principle in the latex (Jatrophone) has shown anti-cancer properties (Kosasi 1989).

There is a need to establish mass multiplication technique to meet the large-scale demand and easy supply of *Jatropha curcas* plant. Macro-propagation through stem cutting is possible but the established plants are not deep rooted, which can be easily uprooted and the seed yields are low too (Heller 1996). Propagation through seeds is dependent on good rainfall, moisture condition, sowing time and depth of sowing. Seed propagated plants are not true to type and are low in oil production. Tissue culture techniques offer rapid and continuous supply of plants. Evaluation of tissue culture propagated *Jatropha curcas* plants revealed that they were at par with seed propagated quality plants in terms of yield and yield related characters (Sujatha and Mukta 1995). Keeping in view the medicinal and bio-diesel importance of *Jatropha curcas* the present study was designed to increase number of plants through micro-propagation by using apical shoot tip explants.

MATERIALS AND METHODS

The experiment was conducted in tissue culture laboratory of Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar. Shoot tip explants 0.5-1 cm were collected from young (2 years old) active growing plants of *Jatropha curcas* grown in PCSIR nursery. The explants collected were washed for an hour under running tap water. Then explants were washed with a detergent for 5 minutes and rinsed 5 times with distilled water to remove dust particles from their surface. Further, the explants were surface sterilized with 0.1% $HgCl_2$ for 4-5 minutes and then rinsed 4 times with sterile distilled

water to remove the $HgCl_2$ particles from explants surfaces before inoculation in the laminar flow hood cabinet.

Culture Media: Murashige and Skoog (MS) (1962) was used as a basal medium. This contained all the nutritional components essential for the growth and development of the cultured tissues. The Medium pH was adjusted at 5.8 before autoclaving. Different phytohormones were added to the basal medium in order to induce shooting or rooting. For shoot induction and multiplication, the basal MS medium was supplemented with either BAP alone or in combination with IBA and NAA. In order to induce rooting in the developed micro-shoots, the basal medium was supplemented with IBA. MS and ½ MS media were used to get the following treatment combinations (Table 1).

Table 1. Concentration of different phytohormones in the shoot induction media used for the micro-propagation of *Jatropha curcas*

Shoot Induction Medium	BAP	IBA	NAA
	Concentration (µM)		
MS	0	0	0
MS+BAP	2.22	--	--
	4.44	--	--
	8.88	--	--
	13.32	--	--
	17.76	--	--
	22.20	--	--
MS+BAP+IBA	4.44	1.23	--
	8.88	2.44	--
	13.32	4.92	--
	17.76	9.84	--
MS+BAP+NAA	4.44	--	1.34
	8.88	--	2.68
	13.32	--	5.37
	17.76	--	10.74

Table 2. Concentration of different phytohormones in the root induction media used for the micro-propagation of *Jatropha curcas*

Root Induction Medium	IBA Concentration (µM)
0.5MS0	0
0.5MS1	4.92
0.5MS2	9.84
0.5MS2	14.76
0.5MS4	19.68

Experimental design and growth environment: The experiment was laid out in Completely Randomized Design (CRD). For shoot induction and multiplication 15 treatments combinations were used as mentioned above. All the cultures were kept under eight hour dark period and sixteen hour photoperiod in incubator. The light intensity was regulated to 2000 lux and the incubator temperature was adjusted at 25 ± 2^oC.

Data collection and Analysis: Shoot induction data were recorded three weeks after inoculation. The micro-shoots developed there were re-cultured for multiplication and data were collected after 6 weeks. The in-vitro produced shoots were cultured again for rooting in rooting media and data on rooting were recorded after 4 weeks. The data recorded were analyzed statistically using Microsoft Excel and statistical software MSTATC (Michigan State University, USA).

RESULTS AND DISCUSSION

Shoot induction and nodal buds formation: Shoots induced from the explants of *Jatropa curcas* L. and shoot buds per explant responded differently to the concentration of Phytohormones (BAP alone or in combination with IBA or NAA) in the basal MS medium (Fig 1). Multiple shoots were observed from the shoot tip explants within three weeks after culturing. Minimum shoot induction and shoot buds were noted from the tips cultured on phytohormone free medium (13.33% and 0.6 shoot buds per explant, respectively). Addition of BAP alone to the basal MS medium enhanced the shoot induction and shoot bud formation. Maximum shoot induction (93.33 %) and shoot buds per explant (3.33) were obtained with 8.88 $\mu M.L^{-1}$ BAP. Further addition of BAP to the culture medium reduced the shoot induction as well as shoot buds per explant in a dose dependent manner (Fig 1A).

Further, the interactive effect of different concentrations of BAP (4.44 to 17.76 $\mu M.L^{-1}$) and auxins (IAA and NAA) on shoot induction and shoots bud formation was also investigated. Addition of BAP and IAA in lower concentration initially inhibited shoot induction. However, additional increase in BAP and IAA concentrations resulted in an increase in shoot induction. Maximum shoot induction (93.33 %) was obtained when 13.32 $\mu M.L^{-1}$ of BAP and 4.92$\mu M.L^{-1}$ IBA were applied (Fig 1B). However, compared with BAP alone, there was an increase in shoot buds per explant with the supplementation of IBA at each BAP concentration and maximum shoot bus per explant (5.5) were obtained when 13.32 $\mu M.L^{-1}$ of BAP and 4.92$\mu M.L^{-1}$ IBA were applied. The NAA application with BAP, however, resulted in decrease in shoot induction but an increase in shoot buds formation. Maximum shoot induction (73.33 %) and shoot buds per explant (5.1) were obtained at 8.88 $\mu M.L^{-1}$ BAP and 2.68 $\mu M.L^{-1}$ NAA (Fig 1C).

The difference in shoot induction percentage and shoot buds per explant may be due to endogenous cytokinins in the explants and culture conditions, as cytokinin promotes chloroplast development, synthesis,

increases cell expansion in leaves and in dicots, increase nutrient sink activity, promotes cell division and organ development (Ross and Salisbury, 1984). The percent shoot induction also indicates explant compatibility to Plant Growth Regulators (PGRs) concentration. As PGRs are responsible for organogenesis so less response at lower concentration may be because of less availability of phytohormones for shooting, and at high concentrations, decreasing response may be due to the toxicity of cytokinins to the multiplying cells. These results are in line with Sarkar *et al.* (2007), who also found 3 mg BAP + 1 mg IBA best combination for shooting in *Jatropha curcas*. However, these are in contrast to findings of Datta *et al.* (2007) who suggested 2.3 µM Kinetin+0.5 µM IBA as best concentration for shooting in *Jatropha curcas*. Similar results were also reported by Datta *et al*. (2007), Sarkar *et al*. (2007) and Shrivastava and Banerjee (2008), who examined the effect of different concentrations of PGRs on shooting from axillary nodal explants of *Jatropha curcas* and found that 3 mg BAP+1 mg IBA was the best combination for shooting in *Jatropha curcas.*

Shoot multiplication and length: Variable multiplication and shoot length response was found when the micro shoots produced during shoot induction stage were sub-cultured at different concentrations of phytohormones (Fig 2). BAP alone increased the number of shoots and shoot length up to 8.88 $\mu M.L^{-1}$ (5.3 shoots per explant and 3.1 cm respectively). Further addition of BAP resulted in a gradual decrease in number of shoots as well as shoot length (Fig 2A).

Similar to the shoot induction and nodal buds formation, a higher shoot multiplication and growth was obtained when BAP was supplemented with IBA or NAA in the medium. However, BAP and IBA combination resulted in the maximum shoot multiplication and length. There was significant enhancement in shoots per culture and shoot length when the MS medium was supplemented with BAP and IBA ($p<0.01$). Maximum shoots per culture (6.7) and shoot length (4.5 cm) were recorded when 13.32 $\mu M.L^{-1}$ BAP and 4.92 $\mu M.L^{-1}$ IBA was added to MS medium (Fig 2B).

Furthermore, significantly positive effect ($p<0.01$) of different concentrations of BAP and NAA was noted on shoots per culture and shoot length. An increase in shoots per culture and shoot length was noted upto 8.88 $\mu M.L^{-1}$ BAP and 2.68 $\mu M.L^{-1}$ NAA. Maximum shoots per culture (5.8) and shoot length (3.4 cm) was obtained at this concentration (Fig 2C). Further increase or decrease in the concentration of these phytohormones resulted in lower number of shoots and shoot length, though still higher than the MS medium alone.

Auxins and cytokinins both are growth promoters and have positive effect on each other. Both must be added to the medium in *in-vitro* growing plants for embryogenesis to occur. If the cytokinins-to-auxins ratio is maintained high, certain cells divide and give rise to others cells that develop into buds, stems and leaves (Ross and Salisbury 1984). In this experiment, maximum shoots per culture as well as maximum shoot length were recorded when BAP (13.32 $\mu M.L^{-1}$) and IBA (4.92 $\mu M.L^{-1}$) were supplemented to the basal medium (MS). This may be due to synergetic effects of cytokinin (BAP) to auxin (IBA). At control (MS medium only) treatment no proliferation was recorded, however, an increase in shoot length occurred. However, when low PGRs concentrations were applied to the basal medium, less proliferation occurred. Shoots generated from nodes of the inoculated shoots as well as from their basal undifferentiated cells. Further studies showed that maximum average shoot length also observed at 13.32 µM BAP+4.92 µM IBA concentration supplementation to the basal medium. At this concentration, maximum shoot multiplication and healthier shoots were recorded, while with further increase in the concentration, shooting as well as shoot length responses decreased. The shoots produced showed stunted growth and browning of leaf tissues, which may be due to the PGRs toxicity towards the multiplying tissues. This could be due to the residual effects of hormones accumulated in the explant tissues by sub-culturing and the use of PGRs, which increase the concentration of endogenous PGRs that causes toxic effects and reverse the growth processes (Ross and Salisbury 1984).

Rooting: The *in-vitro* grown shoots obtained from shoot proliferation stage were transferred to the different rooting media. Initially, MS medium in two concentrations was used to determine the root induction as well as number of roots per explant. There were no significant difference of the full and half concentration of MS medium on root induction or number of roots. Thus half MS medium was used in the further experiments (Fig 3). IBA supplementation has a significant effect on percent root induction as well as number of roots per explant ($P\leq0.01$). 100% rooting occurred in *Jatropha curcas in-vitro* grown shoots, when ½ MS medium was supplemented with either 9.84 or 14.76 $\mu M.L^{-1}$ IBA. Similarly, maximum roots per explant (4.93) were obtained in shoots supplied with 14.76 $\mu M.L^{-1}$ IBA. The root induction as well as root number decreased with higher concentration of IBA.

It is well known that auxins promote adventitious root development on stems (Ross and Salisbury, 1984). Maximum roots per *in-vitro* shoot and root induction were recorded when IBA (14.76 $\mu M.L^{-1}$) was added to ½ MS medium, while with further increase or decrease in concentration of IBA resulted in a decline in root induction as well as number of roots. These results are in contrast to those of Sarkar *et al.* (2007), who reported 2.5 mg IBA for best rooting in *Jatropha curcas*.

However, they are in line with Shrevastava and Banerjee (2008), who recommended 3 mg IBA addition to ½ MS medium for best rooting.

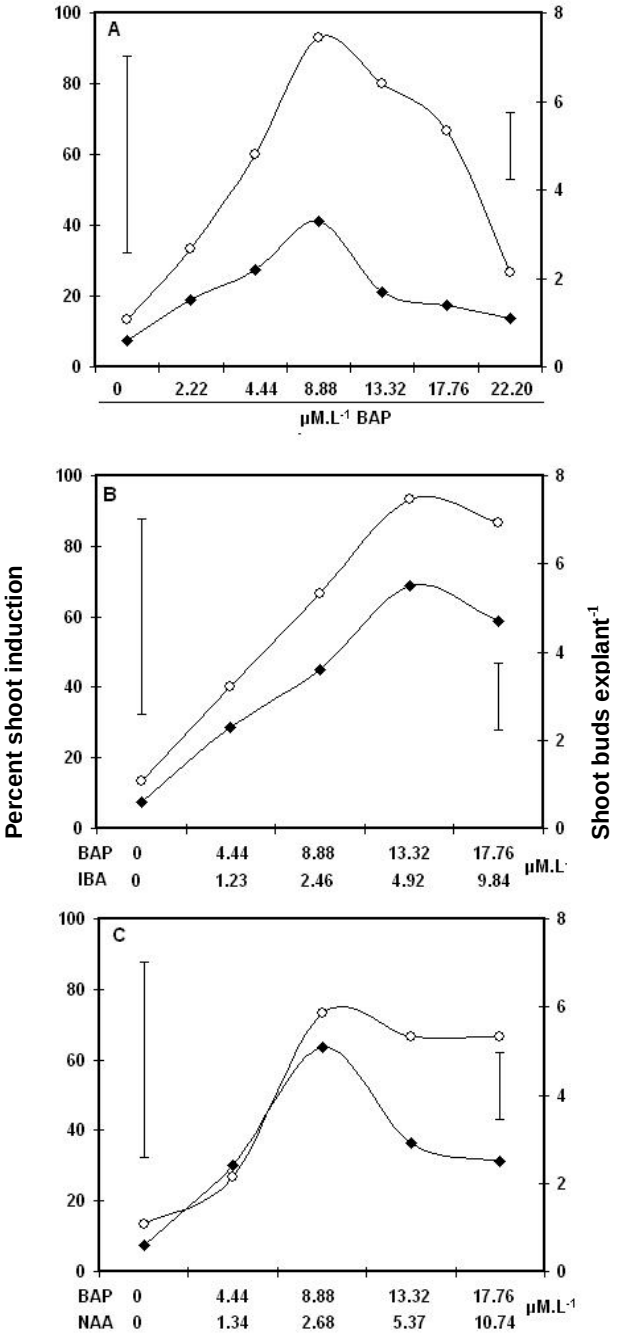

Fig 1. Effect of different concentrations of the phytohormone BAP alone (A) and in combination with IBA or NAA (B and C, respectively) on the shoot induction (○) and number of shoot buds explant^{-1} (♦) of *Jatropha curcas*. The bars represent the LSD values for shoot induction (left) and number of shoot buds explant^{-1} (right).

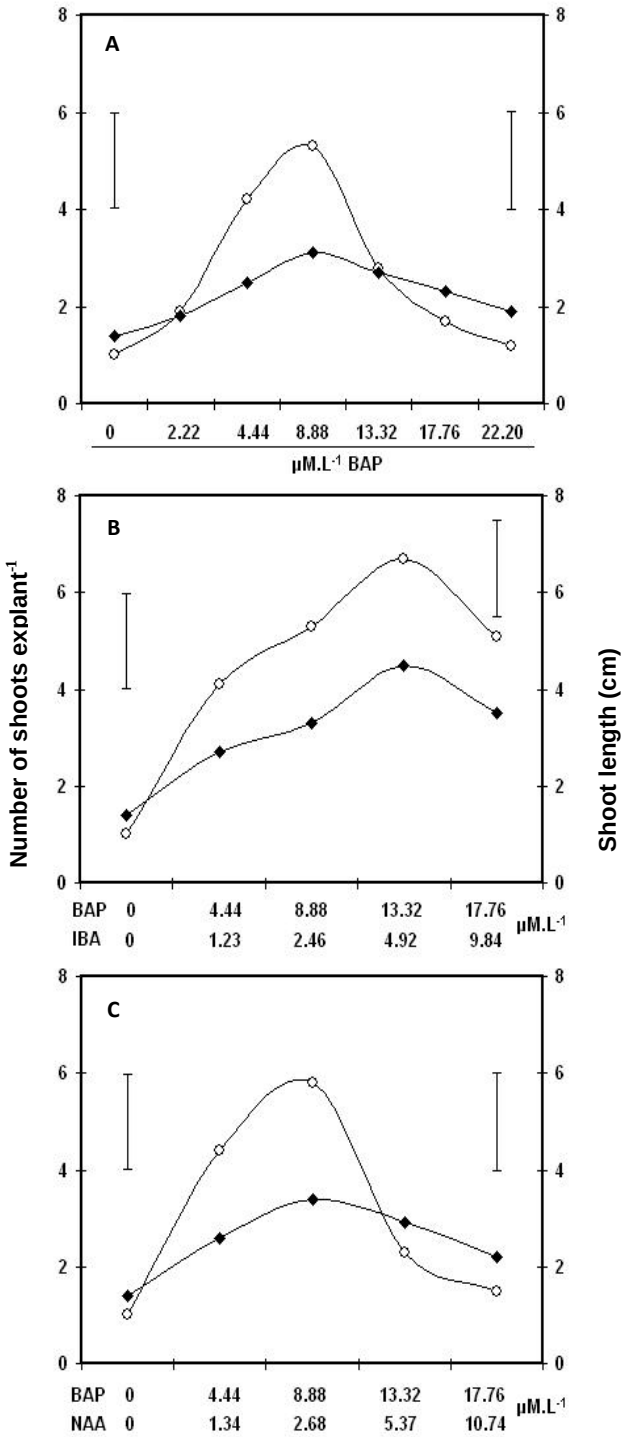

Fig 2. Effect of different concentration of the phytohormone BAP alone (A) and in combination with IBA or NAA (B and C, respectively) on number of shoots explant^{-1} (○) and shoot length (♦) of *Jatropha curcas*. The bars represent the LSD values for number of shoots explant^{-1} (left) and shoot length (right).

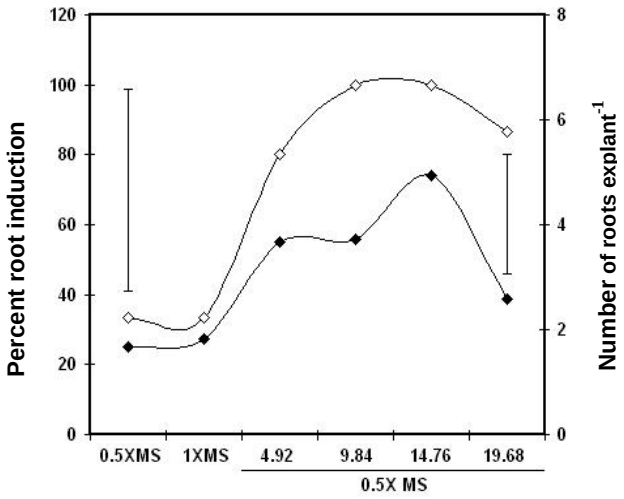

Figure 3: Effect of different concentrations of IBA on root induction (◊) and number of roots explant^{-1} (♦) from *in-vitro* grown shoots of *Jatropha curcas*. The bars represent the LSD values for root induction (left) and number of roots explant^{-1} (right).

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