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ORIGINAL ARTICLE

Comparative Effects of Auxins, Jasmonic Acid and Salicylic Acid on Callus Initiation and Organogenesis in *Vigna mungo* (L.) Hepper Using Hypocotyl Explant

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An attempt has been made to compare the effects of conventional phytohormones like IAA, NAA, BAP to the recent hormones viz., JA and SA in callus induction in blackgram using hypocotyl as explant source. Instead of testing the hormone individually, a combination of hormones was used to induce callus and organogenesis. A significant callusing response was noticed in MS medium supplemented with 0.5 ppm of 2,4-D and 1 ppm of IAA. Likewise, Salicylic acid at 1 ppm induced high percentage of callus induction proving its efficiency in inducing the root formation. Jasmonic acid induced a high percentage of callus induction at 1.5 ppm and greater rooting response than SA. Organogenic callus was observed at JA and SA supplementation. The combination of NAA, BAP and SA showed that 0.75 & 1.5 ppm of NAA, 1 ppm of BAP and 0.5 ppm of SA showed a better response in culture medium. Thus, the combination of these hormones seems serve as efficient growth supplements for in vitro culture of many agricultural crops.

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Plant tissue culture is commonly used to describe the *in vitro* and aseptic growth of any plant part on a nutrient medium. Growth hormone is a natural chemical that exerts strong controlling effects on growth and development. It is used either in low or high concentrations to promote the callus or shoot and root formation. IAA and IBA are generally responsible for root induction whereas BAP and cytokinins are known for shoot induction and 2,4-D for callus induction. Immature cotyledonary node explants produced a high frequency of plant regeneration in several crop species (Tivarekar and Eapen, 2001).

SA acts as a potential non-enzymatic as well as a plant growth regulator, which plays an important role in regulating a number of plant physiological processes (Fariduddin *et al*., 2003; Singh and Usha, 2003; Waseem *et al*., 2006; Arfan *et al*., 2007). JA has been reported to influence a wide variety of physiological and developmental responses

(Parthier *et al*., 1992). The major functions of JA in regulating plant growth include growth inhibition, senescence and leaf abscission. JA has an important role in response to wounding of plants and systemic resistance in plants inhibiting the insect ability to digest protein.

MATERIALS AND METHODS

Explant sterilization and inoculation

Vigna mungo (L.) Hepper seeds were soaked in tap water for 30 minutes. Prior to germination, the healthy seeds were subjected to several steps of surface sterilization procedures as given below: The seeds were washed in distilled water containing a few drops of Tween 20, a wash in 0.1% (w/v) $HgCl_2$ solution and finally under UV-C (Philips, India) exposure for a brief period of 5 mins. The germinated hypocotyls were used as the source of explants. Hypocotyl explants of 1-2 cm in length were excised using a sterile sharp blade in a Laminar air flow chamber (Atlantis, India) and implanted in MS medium supplemented with different concentrations of 2,4-D (0.5-2.0 mg/L) and Salicylic acid (0.5-2.5 mg/L), IAA (0.5-1 mg/L), Jasmonic acid (0.5-2.5 mg/L) and BAP + NAA (0.5-2 mg/L).

RESULTS

Supplementation of 2,4-D in MS medium

The callus formation varied on MS medium supplemented with different growth regulators. Good callusing response was noticed at 0.5 ppm and 0.75 ppm of 2,4-D whereas moderate callusing response was noticed at 1.0 ppm and 1.5 ppm of 2,4-D (Table 1). High concentration viz., 2 ppm showed least callusing response. The supplementation of 2,4-D in MS medium had resulted in induction of brown compact callus (Fig. 1a).

Supplementation of IAA in MS medium

Regarding IAA, 0.5ppm and 0.75ppm showed high callusing response whereas a low callus induction at 2ppm of IAA was noticed (Table 1). High concentration such as 1.5ppm of IAA showed a moderate callusing response by producing a brown compact callus (Fig.1b).

Supplementation of SA in MS medium

The role of SA in the induction of organogenic callus was studied in *Vigna mungo*. All concentrations of SA showed positive response of callus induction (Table 1). But the frequency of callus was 40% at 1 ppm of SA. All concentrations of SA produced well branched roots except that the nature of the root was different at each concentration. At 0.5ppm of SA, thin roots with slightly branching was produced whereas 1ppm of SA produced well branched thick roots (Fig.1c).

SA has been reported to improve *in vitro* regeneration as well as abiotic stress tolerance in plants. The effect of various concentrations of SA or *in vitro* propagation of shoot apices of *Hibiscus* showed the regenerating potential of SA (Sakhanokho *et al*., 2009). SA has been used to enhance *in vitro* regeneration in several other plants species (Quiroz-Figueroa *et al*., 2001; Luo *et al*., 2001; Hao *et al*., 2006). In the present study, SA was found to induce root formation than shoot initiation. The response of root initiation was seen at all concentrations with maximum at 1 ppm. Addition of 0.5 mM SA induced shoot growth with more shoots per explants in *in vitro* culture of *Hibiscus* (Sakhanokho *et al.,* 2009).

Supplementation of JA in MS medium

Like SA, JA also produced organogenic callus. The frequency of root formation was 50% at 1 ppm of JA and maximum response was noticed at 1.5

ppm of JA (Table 1). At 0.5 ppm, JA directly produced roots without undergoing callus formation. At 1.5 ppm of JA, long and well branched roots with 100% frequency of root formation was noticed (Fig.1d). At 2ppm of JA, there was moderate root formation. The effect of JA on shoot formation in cotyledons was examined by Patricia *et al*. (2001). Shoot formation was initiated below 10 µM.

An initial exposure of *Pinus* cotyledons to JA resulted in shoot primordial and subsequent shoot formation (Patricia *et al*., 2001). In our study, JA at 0.5ppm to 2.5ppm stimulated root primordial and subsequent root formation with lateral branches. JA has been reported to influence shoot and bulb formation in garlic at 1–10 µM concentration (Ravnikar *et al*., 1993).

Supplementation of NAA, BAP and SA in MS Medium

A combination of NAA and BAP was tested for callus induction using the hypocotyl explants of *Vigna mungo* (Table 1). The hypocotyl explants of *Vigna mungo* responded at NAA (0.75 ppm) + BAP (1 ppm) + SA (0.5 ppm), NAA (1 ppm + BAP (1 ppm) + SA (0.5 ppm), NAA (1.5 ppm) + BAP (1 ppm) + SA (0.5 ppm), NAA (2 ppm) + BAP (1 ppm) + SA (0.5 ppm). The callus initiation response was high in MS medium supplemented with NAA (0.75 ppm and 1.5 ppm), BAP (1.0 ppm) and SA (0.5 ppm). Combined action of NAA (0.75 ppm) and BAP (1.0 ppm) on inoculated hypocotyl explants induced callus formation which was found to be friable in nature. Addition of SA (at 0.5 ppm) to this combination resulted in root initiation from the callus is shown in (Fig.1e).

High frequency of callus induction was noticed from hypocotyl explant of *Vigna mungo* at 0.75 ppm of NAA with 1 ppm of BAP and 0.5 ppm of SA. Callus growth was highest (70%) when supplemented with IAA (3 mg/l) + BAP (2 mg/l) in *Boerhaevia diffusa* L. (Samiron *et al*., 1999). In the present study, the addition of SA and JA to the MS medium resulted in enhanced callus initiation as well as root initiation. Compared to SA, JA was effective in inducing profuse rooting with lateral branching. Thus, the combination of the phytohormones proved to be beneficial in both shooting and rooting responses in *Vigna mungo*.

Table 1 : Effect of various concentrations of 2,4-D, IAA, NAA, BAP, SA & JA on callus induction from hypocotyl explants of *Vigna mungo* in MS medium (15 days after inoculation)

Hormone Concentration (ppm)	Frequency of callus induction (%)						Nature of callus
	2.4-D	IAA	NAA	BAP	SA	JA	
0.5	80	70	65	55	40	60	Organogenic
0.75	70	80	80	60	60	50	Organogenic
1.0	70	60	60	80	70	70	Organogenic
1.5	60	50	70	75	50	100	Brown compact
2.0	40	40	70	60	40	60	Brown compact

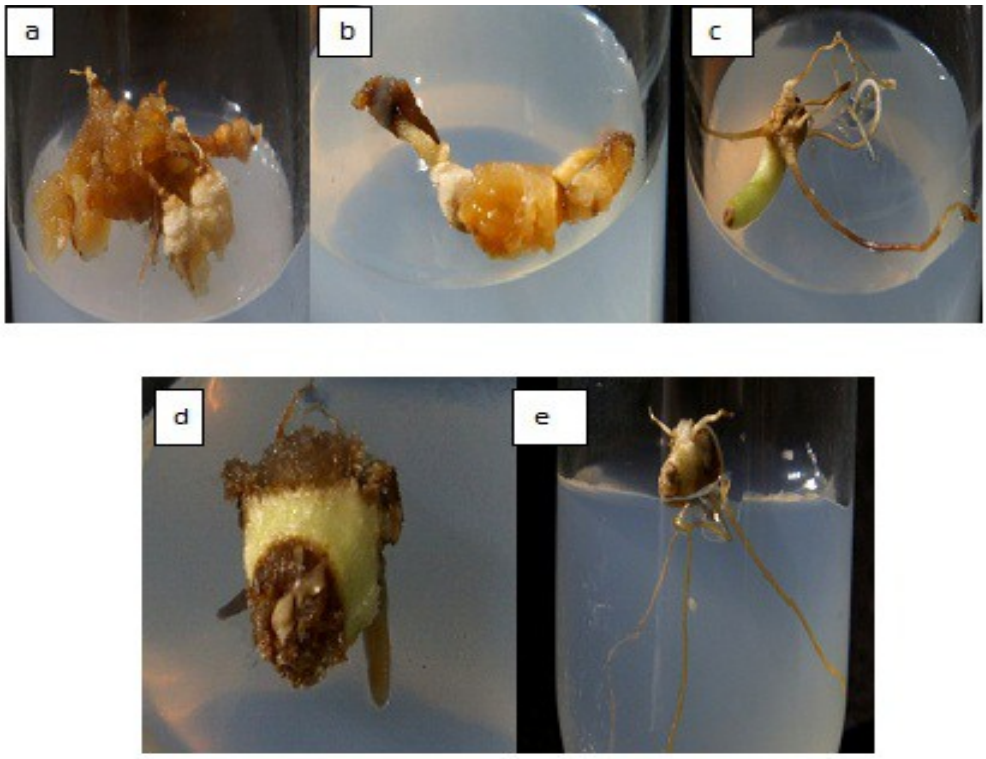

Figure 1 : Induction of compact, organogenic callus and multiple roots from the hypocotyl explants of *Vigna mungo* (L.) Hepper on MS medium supplemented with dififerent concentrations of 2.4-D, IAA, SA, IA, BAP+ NAA, (a, b, c, d and e).

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