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Full Length Research Paper

Regeneration of okra *(Abelmoschus esculentus* L.*)* via apical shoot culture system

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The present study was undertaken to evaluate the most suitable concentration of growth regulators for regeneration of okra *(Abelmoschus esculentus* L. Monech*)* via apical shoot culture system. The study of apical shoot culture system was found effective for regeneration of apical shoots. The okra *(A. esculentus* L. Monech*)* N-550 line evolved at R&D, Nirmal Seeds Pvt. Ltd., was used as basic material for experiment of regeneration using different auxin and cytokinin hormone combination. Nine to ten days old germinating seedlings were used for isolation of shoot tip explants, isolated shoot tips were cultured on regeneration medium, Murashige and Skoog (MS) medium supplemented with alone indole-3-butyric acid (IBA) (0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L) and IBA 1.0 mg/L with naphthalene acetic acid (NAA) (0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L). After regeneration, cultures were inoculated on elongation medium containing MS medium supplemented with kinetin (0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L). Elongated shoots transferred to rooting medium containing MS medium supplemented with indole-3-butyric acid (IAA) (0.25, 0.5, 1.00, 1.5, 2.0 and 2.5 mg/L). Combination of 1.0 mg/L IBA and 0.5 mg/L NAA were found to be most effective for plant regeneration from apical shoot. Best shoot elongation observed in MS medium supplemented with kinetin 0.5 mg/L. Elongated shoots rooted most effectively in MS medium containing 0.5 mg/L IAA and 1.0 g activated charcoal. The success of apical shoot culture system of okra was encouraged by acclimatization of the plantlets in the field conditions.

Key words: *Abelmoschus esculentus*, apical shoot, auxins, cytokinines, regeneration.

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India and many other countries. India ranks first in the world with 3.5 million tons (70% of the total world production) of okra produced from over 0.35 million ha land (FAOSTAT, 2008).

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Abbreviations: MS, Murashige and Skoog medium; **IBA,** indole-3-butyric acid; **NAA,** naphthalene acetic acid; **IAA,** indole-3-butyric acid.

Okra is known by many local names in different parts of the world and commonly called as “Bhindi” in India. Even in India, different names have been given in different regional languages (Chauhan, 1972). It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. India is the largest producer of okra covering an area of 3.6 lakh hectors with an annual production of 34.2 lakh tones. The states Uttar Pradesh, Assam, Bihar, Orissa, Maharashtra, West Bengal and Karnataka are the major producers of okra. Karnataka has an area of 8853 hectors with an annual production of 0.73 lakh tonnes. It is a potential export earner accounting for thirteen per cent among fresh vegetables (Zaheerpasha 2007).

The roots and stems of okra are used for cleaning the cane juice from which gur or brown sugar is prepared (Chauhan, 1972). Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries. Mature

Figure 1. Apical shoot inoculation on MS medium containing IBA + NAA.

fruits and stems containing crude fiber are used in the paper industry. Extracts from the seeds of the okra is viewed as alternative source for edible oil. The oil content of the seed is quite high at about 40%. Okra provides an important source of vitamins, calcium, potassium and other mineral matters which are often lacking in the diet of developing countries (IBPGR, 1990). It is known as powerhouse of valuable nutrients having low calories and is fat-free. It also has considerable medicinal and industrial value (Kirtikar and Basu, 1984). Due to its high economic importance, considerable attention has been paid to improve Okra plants by conventional plant breeding methods. Although this method (interspecific hybridization) has been difficult, advances have been made by using biotechnological techniques such as tissue culture. Tissue culture is a major prerequisite for the production of transformed plants (Mitra, 2011). It is necessary to develop an effective regeneration protocol by a range of different techniques to widen the possibilities for the development of transgenic lines or somaclonal variants of different cultivars.

Callus induced genetic damage is commonly observed among regenerated plants (Stelly et al., 1985; Li et al., 1989). Therefore, development of tissue culture protocols to induce efficient proliferation in a genotype independent manner is desirable for genetic transformation of okra compared with somatic cell culture. Shoot apex culture is an easier method to obtain regenerative plants (Zhang et al., 2000). Theoretically, the advantage of the shoot apex explants over other regeneration systems is that plants may be obtained from any genotype (Zapata et al., 1999a, b).

The present study describes a simple, rapid, efficient and an optimized regeneration system in *A. esculentus* (Okra) by using apical shoot as explant. This is the first report of regeneration of okra using apical shoot as explant.

MATERIALS AND METHODS

Plant material

Seeds of Okra (*Abelmoschus esculentus* N-550) were obtained from Nirmal Seeds Pvt. Ltd., Pachora, Jalgaon, (MS) India. The seeds were sterilized as per the standardized protocol. 7 to 10 seeds per bottle were germinated on 20 ml of full strength Murashige and Skoog (MS) basal medium. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 15 min. Seeds were maintained at room temperature (25 ± 2ºC) at 16 h photoperiod (60 to 80/ mol m^{-2} s^{-1})

Surface sterilization of okra seeds

Healthy seeds were sorted out from the stock of available seeds and washed three times with sterile double distilled water. After washing, seeds were soaked overnight in cool water containing 1% bavistine. On next day, soaked seeds were washed three times with sterile distilled water and treated with 30% H_2O_2 for 30 min. Traces of H_2O_2 were removed by washing with sterile distilled water. 0.01% $HgCl_2$ treatment was given for 30 min and again washed with sterile distilled water to remove traces of $HgCl_2$. Washed seeds were air dried and inoculated on MS full strength medium for germination.

Explants isolation

Nine to 10 days old germinating seedlings were used for isolation of shoot tip explants. The seedling shoot is embedded in the stem between the cotyledons. One cotyledon was removed by pushing down on it until it snaps off to expose shoot apex, then another cotyledon was removed, shoot apex was excised from hypocotyls by cutting at the base of it and inoculated in the medium (Figure 1).

Regeneration

Shoot apices of the cultivar N-550 were placed on MS basal medium supplemented with (vitamin stock) 100 mg/L myo-inositol, 0.1 mg/L thiamine-HCl, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine-HCl, 3% sucrose (Zapata et al., 1999b) and different combinations of plant growth regulators indole 3 butyric acid (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and naphthalene acetic acid (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) were used. The pH of the medium was adjusted to 5.8 before autoclaving. Media was solidified with 8.0 g/L agar-agar. Shoot apices were incubated at 25 ± 2°C under a 16 h photoperiod. For elongation, shoots were transferred to selection media containing Kinetin (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L). After three to four weeks, the responses of plant regeneration and elongation were recorded.

The elongated shoots were transferred to culture bottles containing the same initial medium supplemented with Indole-acetic acid (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and 1.0 g/L activated charcoal for rooting. The rooted shoots were finally planted in soil in small cups, watered and kept under observation in growth room conditions.

Then rooted shoots were planted in pots and are transferred to the green house for further acclimatization.

Table 1. Effect of different concentration of IBA on percent shoots regeneration response and shoots length in shoot tip culture of okra when used alone.

Concentration of IBA (mg/L)	Number of shoot regeneration (%)	Length of shoot regeneration (cm)
0.25	58.0	1.00
0.50	59.0	1.10
1.00	82.0	2.30
1.50	62.0	1.20
2.00	68.0	1.15
2.50	67.5	1.20

Table 2. Effect of different concentration of NAA with IBA on percent shoot regeneration response and shoot length in shoot tip culture of okra.

Concentration of NAA (mg/L)	Concentration of IBA (mg/L)	Number of shoot regeneration (%)	Length of shoot regeneration (cm)
0.25	1.00	89.0	2.50
0.50	1.00	98.5	3.50
1.00	1.00	92.0	2.90
1.50	1.00	91.0	2.60
2.00	1.00	90.5	2.55
2.50	1.00	93.0	2.40

Figure 2. Regeneration of apical shoots on MS medium containing IBA + NAA.

RESULTS

In the sterilization process of seeds it was observed that prolong treatment of 0.01% $HgCl_2$ that is for 30 min reduces the level of contamination. Similar results were also observed by other workers (Munjury, 1998; Banu, 1998; Das et al., 2001) with this treatment.

Plant regeneration

Organogenesis of explants was initiated by the effect of different growth regulators. When apical shoots were cultured on MS medium supplemented with alone indole-3-butyric acid (IBA, 1.00 mg/L) (82%) shoot regeneration with shoot length of 2.30 cm was recorded (Table 1) however using IBA with different combinations of naphthalene acetic acid (NAA), the highest shoot regeneration (98.5%) along with shoot length 3.5 cm was observed in MS medium supplemented with IBA (1.00 mg/L), NAA (0.50 mg/L) (Table 2 and Figure 2). In elongation medium efficient plantlet growth 99.0% along with shoot length of 5.6 cm was observed in MS medium supplemented with kinetin (0.50 mg/L) (Table 3 and Figure 3). Introduction of roots on regenerated shoots are essential for successful establishment of the plantlet on the soil. In apical shoot derived plantlets, highest frequency of rooting (99%) having root length 5 cm were recorded in MS media containing indole-3-acetic acid (IAA, 0.50 mg/L) and charcoal 1 g/L (Table 4 and Figure 4).

DISCUSSION

Apical shoot regeneration is direct, relatively simple and needs less time to regenerate large number of plants. Plants regenerated from shoot apices are true to phenotype with low incidence of somaclonal variation and chromosomal abnormalities (Bajaj, 1998). Apical shoot as explants are considered as more appropriate because

Table 3. Effect of different concentration of Kinetin on percent shoots elongation response and shoots length in shoot tip culture of okra when used alone.

Concentration of kinetin (mg/L)	Number of shoot elongation (%)	Average shoot length (cm)
0.25	82.0	4.80
0.50	99.0	5.60
1.00	89.0	4.95
1.50	91.0	4.00
2.00	92.5	4.20
2.50	91.0	4.10

Table 4. Effect of different concentration of Indole-3-acetic acid (IAA) on percent rooting response and root length in shoot tip culture of okra when used alone.

Concentration of IAA (mg/L)	Number of explant root regeneration (%)	Length of root elongation (cm)
0.25	69.5	3.0 ± 1
0.50	78.0	3.5 ± 1
1.00	99.0	5.0 ± 1
1.50	92.0	4.5 ± 1
2.00	93.0	4.0 ± 1
2.50	92.5	4.3 ± 1

Figure 3. Elongation of regenerated shoots on MS medium containing Kinetin.

Figure 4. Rooting on MS medium containing IAA+ Charcoal 1.0 g/L.

meristematic cells are programmed for apical shoot regeneration (Zapata et al., 1999b). The necessity of cytokinin for shoot initiation is well established (Beck and Caponetti, 1983; Evans et al., 1984). It is generally believed that hormonal balance is a key factor in regulating morphogenesis in explants (Satyavathi et al., 2002; Ebrahimi et al., 2006). The balance and ratios of hormones present is what helps to influence plant reactions: it possibly regulates enzymatic reactions in the plant by amplifying them, influencing plant growth results. Hormone balance is apparently more important than the absolute concentration of any one hormone and is presumably important to the overall effect on growth and morphological changes. Our result indicates that the regeneration and elongation of apical shoot was promoted by a balance of cytokinin and auxin.

In the present study hormone combination of IBA 1.0

mg/L and NAA 0.5 mg/L in MS medium were found better for apical shoot regeneration (98.5%) than IBA 1.0 gm/L (80.0%) alone. Elongation of regenerated apical shoot was found to be 5.60 cm in MS medium, supplemented well with kinetin 0.5 mg/L (99%). Introduction of roots on elongated shoots are essential for successful establishment of the plantlet on the soil. In apical shoots, highest frequency (99.0%) of rooting was recorded in media containing 1.0 mg/l IAA (Table 4).

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