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Full Length Research Paper

Optimization of medium conditions for efficient plant regeneration from embryo of cotton (var.narishima)

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An efficient and rapid regeneration protocol was developed using embryo from germinating seedlings of cultivar of cotton (var.narishima) as an explant. A vertical slit given in the apex of embryo shoot regeneration was successful on murashige and skoog medium with 2 mg/L benzyl amino purine (BAP). The shoots were sub cultured in every 15 days in regeneration medium and later they were transferred to rooting medium (1 mg/L IBA).

Key words: Cotton, embryo, tissue culture, regeneration, plant growth regulators.

INTRODUCTION

Cotton is an important crop that is grown throughout the world. It is grown as a source of fiber, food and feed. Lint, the most economically important product from the cotton plant, provides a source of high quality fibre of the textile industry. Cotton seed is important source of oil and cotton seed meal is a high protein product used as livestock feed. Although significant progress has been made in the field of cotton improvement with conventional breeding methodology with the limitations to introduce new alleles. Genetic engineering offers a directed method of plant breeding that selectively targets one or a few traits for introduction into the crop plant. Over 100 years ago, Haberlandt envisioned the concept of plant tissue culture for the cultivation of plant tissues and organs. Plant tissue culture is now a well established technology. Around the mid of twentieth century, there is a notion that plants could be regenerated or multiplied from either callus or organ culture through either micropropagation or clonal propagation. But today, these routine technologies have been expanded to include somatic embryogenesis, somatic hybridization, virus elimination as well as the application of bioreactors to mass propagation. The first transgenic cotton plants were reported by Umbeck et al. (1987). Since then many researchers have been reported insect resistant (Perlak et al., 1990; Cousins et al., 1991; Thomas et al., 1995; Li et al., 1998) or herbicide resistant (Bayley et al., 1992; Rajasekharan et al., 1996; Keller et al., 1997) transgenic cotton plants. Transgenic cotton has been mainly obtained by *Agrobacterium*-mediated transformation (Satyavathi et al., 2002) and also been generated by regeneration from shoot apex tissues (Gould and Magallanes-Cedeno, 1998). Another method followed to regenerate transgenic plants in cotton has been the pollen tube pathway transformation. In this paper we report on generating cotton transgenic plants by regeneration from embryo of var.narshima, a popular variety in most of the cotton growing areas in India.

To tackle the problems pertaining to regeneration in cotton and certain other recalcitrant crops, alternate methods to minimize or eliminate the steps of regeneration are being standardized. These are called in planta transformation protocols. The strategy essentially involves in plant inoculation of embryo axes of germinating seeds and allowing them to grow into seedling *ex vitro.* Research with Arabidopsis has benefited from the development of high throughput transformation method that avoid plant tissue culture (Azipiroz-leehan and Feldmann, 1997).

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Abbreviations: MS, Murashige and Skoog's medium; **BAP,** benzyl amino purine; **IBA,** Indole butyric acid.

MATERIALS AND METHODS

Source of seeds

The seeds of cotton (var.Narasimha) were procured from

Table1. Effect of various concentrations of BAP with MS on shoot regeneration from embryo apex explant in cotton.

Treatments	BAP concentration (mg/L)	Cultures showing shoot regeneration (%)	No. of shoots per culture	Shoot vigour
MS+ T1 (control)	0BAP	ND	ND	ND
MS+ T2	1BAP	12±1.27	2.3	Poor
MS+ T3	2BAP	90±8.82	18.3	Good
MS+ T4	3BAP	70±6.67	16.2	Good
T5	MS+4%BAP	50±3.33	10.4	Moderate

Figure 1. (a) Embryo explants; (b)shoot regeneration and sub-culturing of embryo explant (After 20 days from embryo); (c) *in vitro* rooting (After 32days from embryo) (d) Regenerated plantlet growing in pots (After 45 days from embryo).

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Seeds of breeding line of cotton which is Narshima (Female) were soaked overnight in distilled water and were surface sterilized first with 1% Bavastin for 10 min and thoroughly washed with distilled water. Later the seeds were transformed to laminar air flow and treated with 0.1%$HgCl_2$ for 10 to 15 min and washed thoroughly with distilled water for three to four times after treatment with each sterilant. This confirms the removal of mercuric chloride (sterilant). The seeds are soaked in distilled water overnight and water is removed. Then seeds are transformed to petri plates where seeds are pressed with forceps so that embryo is pushed out. Embryo was split longitudinally to about half of its length from the top. The explant was placed in contact with the medium. The medium used was MS containing, 3% sucrose, 0.8% agar with different concentrations (1,2,3,4 mg/L) of the plant growth regulator, Benzyl amino purine (BAP) were tested. The culture was maintained in dark for two days and transferred to light (85 µmol m^{-2} s^{-2}) at 25°C±1°C for 15 days with one sub culture was maintained for every 15 days thereafter. Observations and regeneration frequencies, number of shoots and shoot length were recorded 15 days after transferring to regeneration media. Elongated shoots were rooted on MS+IBA (indole butyric acid) 1 mg/L+3% sucrose+0.8% agar. Plantlets transferred to plastic buckets containing soil rite were irrigated with water and/or half strength Hoagland solution alternatively. For histological studies, embryo were fixed in acetic acid and alcohol (3:1) for 24 h, stained with hematoxylin and viewed under Olympus CX31 light microscope.

RESULTS, DISCUSSION AND CONCLUSION

The type and concentration of plant growth regulators strongly influenced the organogenic potential of the embryo apex explant of variety Narshima. The responding frequency of embryo seemed to depend more on concentration of benzyl amino purine. The regeneration response of embryo apex explant in cotton to the concentration of BAP is shown in Table 1. The regeneration of cotton plant from embryo at different stages are shown in Figure 1. It is evident that without

BAP there is no response of regeneration and maximum shoot regeneration response (90±8.82) has been observed with 2 mg/L BAP concentration along with MS. The further increment in BAP concentration to 3 and 4 mg/L along with MS showed decreased shoot regeneration response of 70±6.67, 50±3.33 respectively. Maximum number of shoots has been recorded (18.3) against the BAP concentration 2 mg/L compared to control (2.3).

Our work demonstrated the role of medium preparation and conditions for efficient regeneration of cotton plant from embryo. This method is cost-effective and successful in commercial perspective.

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