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Rapid *In vitro* Root Induction in Transgenic Cotton Shoots

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An efficient *in vitro* rooting technique was developed for recovery of transgenic cotton plants. The loss of transgenic shoots due to failure to form roots is genotype dependant and represents a significant limiting factor in the overall recovery of transgenic plants from cultures. Transgenic shoots following selection on antibiotic medium were efficiently rooted on MS containing different combinations of kinetin, IBA and IAA. Healthy and efficient rooting was achieved when the shoots with blackish and dead root portion were treated with 1.0 mg/ml IBA and cultured on MS medium containing 2% sucrose. This method for *in vitro* rooting of cotton shoots proved to be a simple and reliable allowing 98% recovery of non-rooting shoots from culture. All the rooted plants normally survived in soil and flowered.

Cotton crop has been difficult to manipulate with high efficiency since the tissue culture methods used for regenerating transgenic plants by indirect transformation via callus. So, *in vitro* regeneration by somatic embryogenesis is limited to a few cultivars (Price and Smith 1979, Davidonis and Hamilton 1983, Shoemaker et al. 1986, Trolinder and Goodin 1988a, Finer 1988, Firoozabady and DeBoer 1993, Gawel and Robacker 1990, Rajasekaran et al. 1996, Hazra et al. 2000, Mishra et al. 2003, Sakhanokho et al. 2004 and Haq 2005). Tissue culture is not only a theoretical prerequisite for plant transformation, but it is employed in almost all transformation systems to achieve a suitable efficiency of gene transfer, selection, and regeneration of transformants.

Rooting in transgenic cotton plants is a major limiting factor, so whatever be the transformation system used, all methods ultimately depend on root formation for the recovery of plants from culture (Luo and Gould 1999). Genotype, position of explants, components of medium and proportion of phytohormone will influence plant regeneration. In the present findings, the root portion in transgenic shoots after selection on antibiotic medium was getting black and ultimately shoots died. In this report a more efficient and simple

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procedure for rooting of transgenic cotton plants on the tissue culture media has been described. We made modifications to the procedures (Gould et al. 1991, Hemphil et al. 1998, Kumria et al. 2003, Sakhanokho et al. 2004 and Ouma et al. 2005). Modifications were related to the growth hormone regime, sucrose level and the procedure to culture the plantlets at rooting stage.

Different concentrations i.e. 1, 1.5, 2, 2.5 and 3% of sucrose were added to MS medium. Plant growth regulators were added to the medium in combinations or separately as Kn (1 mg/l), IAA (1 mg/l), and IBA (1 mg/l). Then pH was adjusted to 5.7 - 5.8 and medium was sterilized at 121ºC and 15 lbs psi for 20 min. Transformed shoots of cotton variety CIM-482 with black and dead root portion were cultured on this modified medium. Another modification was made that the dead and black root portion was cut with a surgical blade and the base of the shoot was just dipped in solution of IBA (1 mg/l) and then cultured on simple MS. The cultures were kept at 27 ± 2°C with a photoperiod of 16 h under the light regime, 100 - 120 μm m^2/s.

Rooted shoots were taken out of the culture vessel and the medium was removed by washing roots with water. The root portion was dipped into solution of IBA (1 mg/l) and planted into the soil pots (Rashid et al. 2004). Non-transformed plantlets were shifted to soil without IBA treatment. Pots were covered with polythene bags after adding 20 - 25 ml of nutrient solution and kept at 30 ± 2°C for 16 hr photoperiod in light intensity of 250 - 300 μmol/m^2/s and removed completely after acclimation of plants.

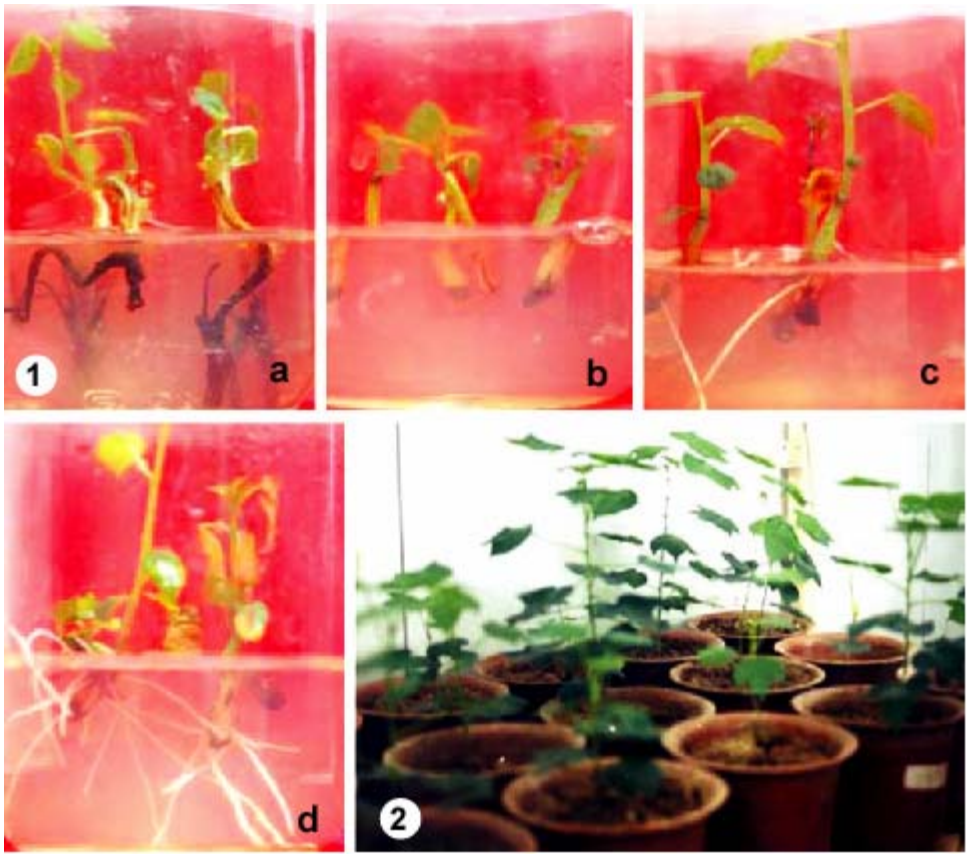
Table 1. Effect of growth hormones on rooting of transgenic shoots.

Growth hormones (mg/l)	Rooting (%)
MS + Kn (1.00) + (IAA 1.00)	45
MS + Kn (1.00) + IBA (1.00)	77
MS + IAA (1.00)	52
MS + IBA (1.00)	80
IBA 1.00 (Black root cut and treated with IBA)	98
MS (Transgenic)	0
MS (Non-transgenic)	92

Non-transgenic plants formed 92% roots on MS. IBA alone or in combination with Kn showed rooting in 80 and 77%, respectively. IAA is a weak auxin and formed 45 and 52% rooting in combination with Kn or alone (Table 1). When dead and black root was cut and treated with IBA solution and again cultured on MS, it formed 98% rooting within one week (Fig. 1). Treating roots with IBA before shifting to soil helped the plants establish well into the soil. Similar

technique with some modifications was used by Hemphil et al. **(**1998**)**. So, to achieve rooting of the transgenic plants IBA is the best treatment alone or in combination with other hormones.

Sucrose at 2% with the combinations of growth hormones produced 95% rooting. Low rooting was obtained on 3% sucrose even with the combinations of growth hormones. Most of the reports suggested lowering the concentrations of sucrose or glucose than the standard MS medium i.e. 3% at the time of rooting (Smith et al. 1977, Davidonis and Hamilton 1983, Shoemaker et al. 1986, Ouma et al. 2004, Sakhanokho et al. 2004). Plants with well developed roots survived



Figs.1-2: 1. Rooting of transgenic cotton plants. (a) Black roots in transgenic shoots. (b) Roots treated with IBA (1 mg/l). (c) Root formation started after IBA treatment. (d) Well developed roots after 10 - 15 days. 2. Transgenic cotton plants were kept at 28 - 30°C, under light intensity of 250-300 μmol/m²/s and humidity 60%.

normally into the soil, flowered normally and fertile seeds were collected (Fig. 2). By this protocol the overall transformation process was reduced from 1 year (Firoozababy et al. 1987, Umbeck et al. 1987) to four - five months (Jin et al. 2005).

The sucrose concentrations at 1, 1.5, 2, 2.5 and 3 % showed 30, 52, 95, 61 and 00 percentage of root development, respectively in transgenic shoots.

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