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Full Length Research Paper

Effect of genotype, explant source and medium on *in vitro* regeneration of tomato

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For the present investigations, five cultivars including PKM-1, Moneymaker, Microtom, Micro-MsK and White Cherry of tomato (*Lycopersicon esculentum* Mill.) were selected. The explants cotyledon, hypocotyl and leaf of all the cultivars of tomato were cultured on MS medium fortified with different concentrations of Benzyl amino purine (BAP) (1.0 to 3.0 mg/L) and 0.1 mg/L indole-3-acetic acid (IAA). Adventitious shoot buds were induced at the cut ends of the explants after three weeks of culture. Adventitious shoots were induced from hypocotyl, cotyledon and leaf explants on MS medium fortified with 1.0 mg/l BAP + 0.1mg/l IAA (Medium A); 2.0 mg/l BAP+0.1 mg/l IAA (Medium B) and 3.0 mg/L BAP + 0.1 mg/L IAA (Medium C). Of the five genotypes tested, PKM-1 showed highest number of shoots per explant followed by Micro-MSK, Microtom and Money maker on MS medium augmented with different concentrations of growth regulators used (A, B & C). The lowest regeneration efficiency was observed from all the explants used in White Cherry. More number of adventitious shoots was induced from leaf explants compared to cotyledon and hypocotyl explants in all the genotypes of tomato studied. Among the three media tested, medium'B' showed superiority in cotyledon and leaf explants compared to medium 'A'. Whereas medium 'C' had shown poor response on regeneration ability of all the explants tested irrespective of genotypes. *In vitro* rooting was achieved on MS medium augmented with 0.1 mg/L NAA (Naphthalene acetic acid) in all the genotypes. Thus the plant regeneration was found to be influenced by the genotype, explant and type of medium.

Key words: Tomato, cotyledon, hypocotyl, leaf, regeneration, plantlet formation.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a highly nutritive vegetable crop. Though it is a temperate crop plant, it is extensively cultivated in the tropical and subtropical regions of the world round the year. It ranks third among vegetable crops (only next to potato and sweet potato) with an annual production of 283 million metric tonnes in the year 2009 (FAO statistical database, 2011). Tomato is rich in vitamin A and C and fibre, and is also cholesterol-free (Hobson and Davies, 1971). Tomato contains approximately 20 to 50 mg of lycopene/100 g of fruit weight (Kalloo, 1991) which is the most powerful antioxidant in the carotenoid family and it protects humans from free radicals that degrade many parts of the body and is also known to prevent cancer (Block et al., 1992; Gerster, 1997).

Due to its importance as vegetable and medicinal value, many laboratories are doing research on tomato to manipulate the nutrient quality through transformation studies. Intravarietal differences in in vitro tomato regeneration from various explants are highly pronounced (Ohki et al., 1978; Frankenberger et al., 1981a, b; Kurtz and Lineberger, 1983; Plastira and Perdikaris, 1997). Specific plant growth regulator requirements for each genotype make the tissue culture task in tomato quite difficult and researchers need to deal with each genotype individually (Bhatia et al., 2004). Successful coupling of a regeneration system and gene transfer procedures will assist in addressing both basic and applied research issues (Bhatia et al., 2005). For genetic transformation, the prerequisite is to develop regeneration protocol. The plant regeneration in tomato is genotype, explant and media dependent. A good in vitro regeneration system is

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essential for an effective genetic transformation for commercial applications. Since genotype, explants and media influence the efficiency of regeneration system, we have undertaken the present studies to evaluate genetically diverse cultivars of *L. esculentum* including PKM-1, Money Maker, Microtom, Micro-MSK and White Cherry for plant regeneration from hypocotyls, cotyledon and leaf explants.

MATERIALS AND METHODS

Seeds of tomato (*L. esculentum*) cvs. PKM-1, White Cherry, Moneymaker, Microtom and Micro-MSK were soaked under running tap water for 24 h. These seeds were sterilized with a mixture 10% NaOCl and 2% SDS (10% w/v) for 3 min. Later the sterilized seeds were washed thrice with sterile distilled water and were dried on sterile tissue paper. The sterilized seeds were germinated aseptically on MS basal medium (Murashige and Skoog, 1962) solidified with 0.8% (w/v) agar (Sigma Chemical Co, USA) in 250 ml Erlenmeyer cnical flasks (ca.50 mlmedium/flask). Cotyledon (0.6 to 0.8 cm^2) and hypocotyl (0.5 to 0.8 cm long) explants from *in vitro* grown seedling (8 to 10 days old) were excised, whereas the leaf explants (1.0 cm^2) were used from three week old axenic seedlings.

Culture media and culture conditions

The hypocotyl, cotyledon and leaf explants were cultured on MS basal medium supplemented with 1.0 mg/L BAP + 0.1 mg/L IAA (Medium A); 2.0 mg/L BAP + 0.1 mg/L IAA (Medium B) and 3.0 mg/L BAP + 0.1 mg/L IAA (Medium C). The media were adjusted to pH 5.8 either with 0.1 N HCl or 0.1 N NaOH before addition of 0.8% (w/v) agar and autoclaved at 121°C under 15 lbs for 15 to 20 min. The media were dispensed into different petriplates and 15 explants were inoculated in each petriplate. All the cultures were incubated at 25°C under 16/8 h (dark/light) photoperiod with light intensity 40 to 50 µmol $m^{-2}s^{-1}$ provided by cool white fluorescent lights (Phillips Ltd). Explants were sub-cultured on the same fresh medium for every four weeks for further proliferation.

In vitro rooting and acclimatization

Micro-shoots (2 to 3 nodes) developed from hypocotyl; cotyledon and leaf explants were excised and transferred on to MS medium fortified with 1.0 mg/L NAA. *In vitro* rooted plantlets were taken out from the culture tubes and washed with sterile distilled water to remove the remains of medium. These were transferred to plastic cups containing sterile vermiculite and covered with polythene bags to maintain the RH (70 to 80%) and later shifted to green house. The acclimatized plantlets were transferred into field.

Data analysis

Data of 15 explants were recorded after four weeks of culture. Each experiment was repeated at least twice with similar results and the data of one representative experiment presented here are the mean value alone and pooled from three explants, five genotypes and three media (A, B and C). Trifactorial experiments were carried out to analyze the effect of genotype, explant and medium and their interactions singly and in combination, on *in vitro* shoot regeneration (number of shoots/explant). Results were analyzed by using ANOVA. Fischer's LSD test (Least Significant Difference) was applied to show statistical significance of difference among the means

Cytological studies

Root tips of about 100 plants regenerated from different explants (pooled from all media tested) were randomly selected developed from three different media were fixed in aceto-alcohol (1:3) mixture. They were treated with 1 N HCl (1 to 2 min) followed by 2% aceto-orcein for about 2 h and then squashed with a drop of 45% acetic acid and observed under a Nikon compound research microscope.

RESULTS AND DISCUSSION

Shoot buds induction and proliferation were found after 10 to 12 days of culture from cut ends of the explants. Data on the average number of shoots formed per explant (hypocotyl, cotyledon and leaf) on different regeneration media; including mediums A, B and C are summarized in Table 1. The cultivar PKM-1 showed the highest number of shoots per explant of all the genotypes tested in the present investigations. Similarly cotyledon explants have responded well on all the media used in contrast to hypocotyl and leaf explants of all the cultivars.

Adventitious shoot buds induction was found to be higher in mediums B and C compared to medium A in all genotypes tested irrespective of explants used. The cv PKM-1 showed the maximum frequency number of adventitious shoots formation in mediums A and B from leaf explants. The cultivar MicroMSK showed better regeneration ability in comparison to Microtom. Money Maker exhibited the maximum frequency of adventitious shoots formation when leaf and cotyledon explants were cultured in mediums A and B. The lowest frequency number of multiple shoots formation per explant was observed from all the explants cultured in White Cherry compared to rest of the genotypes used (Figure 1).

Medium B showed more number of shoots formation in all the explants cultured. Leaf explants of all the genotypes have responded well and produced more number of shoots. However cotyledon explants showed superiority over hypocotyls (Figure 2). It is interesting to note that leaf explants showed efficient regeneration on medium B which suggests that application of leaf as an explant source can minimize the variation brought by the genotypes (Figure 3). Evaluation of three media for shoot regeneration (pooled from 5 genotypes) revealed that medium B is superior to the other combinations of media (Figure 4). When explant and medium interaction for optimal shoot regeneration evaluated, it was observed that leaf explants regenerated maximum on mediums B and C, whereas cotyledon and hypocotyl explants on mediums A and B (Figure 5). Similarly, the literature showed that the combination of IAA + BAP for shoot regeneration from different explants of tomato was found to be more effective (Duzyman et al., 1994; Selvi and Khader, 1993; Villiers et al., 1993; Chandel and Katiyar, 2000; Gunay and Rao, 1980; Kurtz and Lineberger, 1983; Chen et al., 1999). However, Pongtongkam et al. (1993), Plastira and Perdikaris (1997) and Venkatachalam et al. (2000) found that BA alone was more effective than IAA +

Table 1. Comparison of the average number of shoot formed per explant on MS medium supplemented with BAP 1.0 mg/L + 0.1 mg/L IAA (A), BAP 2.0 mg/L + 1 mg/L IAA (B) and BAP 3.0 mg/L + 0.1 mg/L IAA (C) in five genotypes of *L. esculentum*.

Genotype	Medium	Explant		
		Hypocotyl	Cotyledon	Leaf
PKM-1	A	5.4^{a}	13.3^{b}	14.6^{b}
	B	4.8^{a}	8.1^{b}	8.3^{a}
	C	4.3^{b}	6.2^{a}	6.7^{b}
Money Maker	A	4.3^{b}	5.5^{b}	7.6^{b}
	B	4.8^{a}	7.2^{a}	10.3^{a}
	C	NR	5.4^{a}	7.0^{a}
Micro-Tom	A	2.4^{b}	4.7^{b}	9.3^{b}
	B	2.5^{NS}	7.2^{b}	14.1^{a}
	C	2.4^{NS}	3.2^{a}	6.5^{a}
Micro-Msk	A	4.1^{b}	9.0^{a}	11.1^{a}
	B	4.9^{a}	7.7^{b}	14.2^{b}
	C	4.4^{b}	9.4^{a}	9.8^{a}
White Cherry	A	NR	4.2^{b}	7.1^{b}
	B	4.6^{b}	6.4^{a}	9.6^{b}
	C	NR	4.0^{b}	5.3^{a}

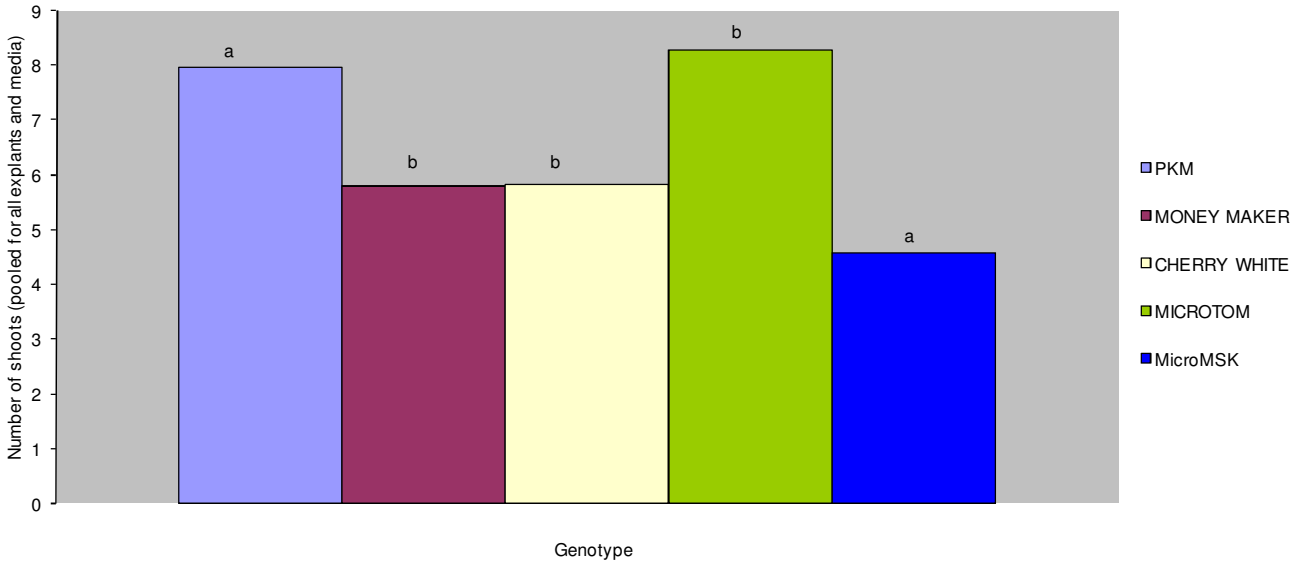

Figure 1. Number of shoots (pooled for three explants and three media) regenerated from *L. esculentum* after 4 weeks of culture. Bars with different letters represent significantly different means ($p<0.01$) using Fischer's LSD procedure.

BA combination for shoot regeneration from leaf, cotyledon and hypocotyl explants. According to our present investigation on five genotypes, we suggest that the growth regulator concentration varies from genotype to genotype and genotypic specific optimal medium concentration is required.

The present protocols are useful for genetic transformation studies in all these five genotypes of tomato.

In vitro rooting and plantlet establishment

Adventitious shoots developed from different explants were excised (3 to 4 cm) and shifted on to root induction medium (MS + 0.1 mg/L NAA). Profuse rooting was observed within two weeks of incubation in all the cultures tested. The *in vitro* rooted plantlets were taken out from the culture tubes and washed to remove adhered

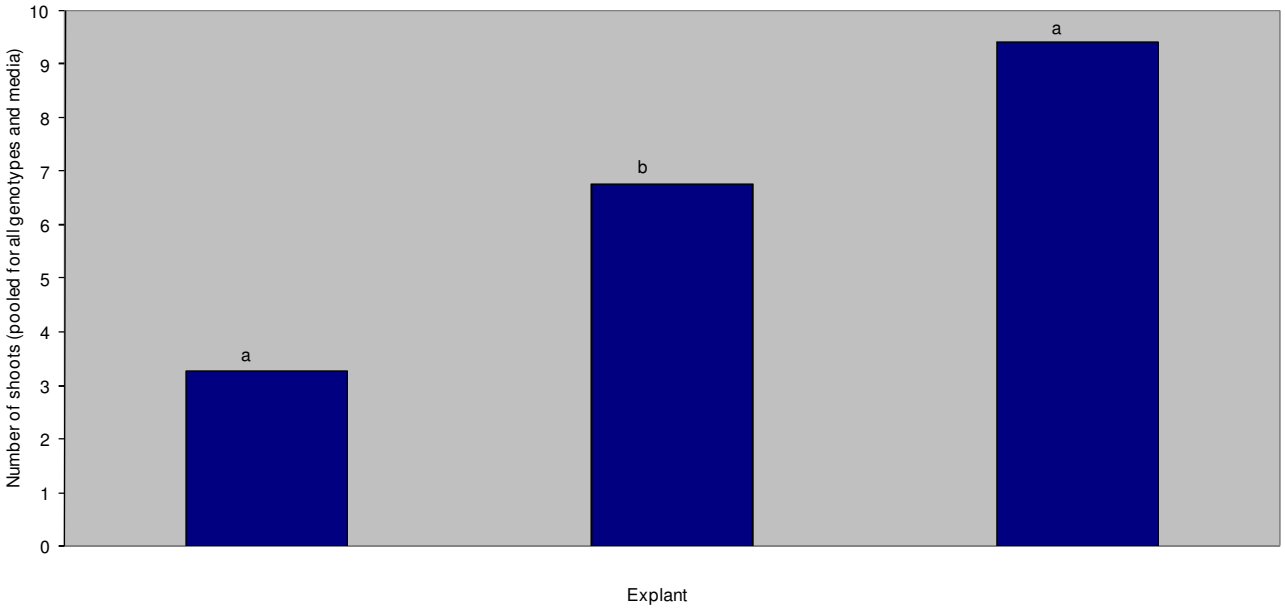

Figure 2. Number of shoots (pooled for five genotypes and three media) obtained from hypocotyl, cotyledon and leaf explants of *L. esculentum* scored after 4 weeks of culture. Bars with different letters represent significantly different means ($p<0.01$) using Fischer's LSD procedure.

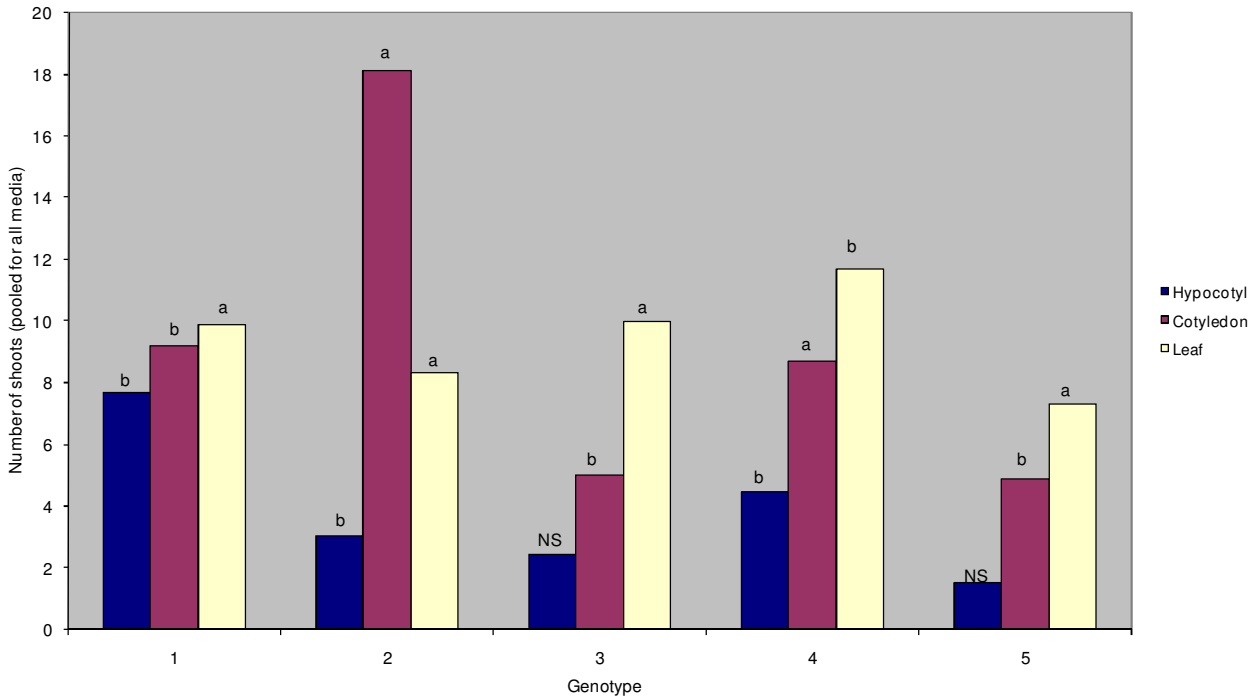

Figure 3. Number of shoots (pooled for three media) obtained from hypocotyl, cotyledon and leaf explants of *L. esculentum* after 4 weeks of culture. Bars with different letters represent significantly different means ($p<0.01$) using Fischer's LSD procedure.

agar and traces of medium. Plantlets were then transferred to plastic cups containing sterile vermiculite. The hardening was done for 4 weeks in a growth chamber and later moved into green house and transferred to field. The survival percentage of plantlets after transplantation was 72% and all the plants developed *in vitro* were found to be morphologically similar to parental plants. Cytological studies showed that the plants regenerated from various

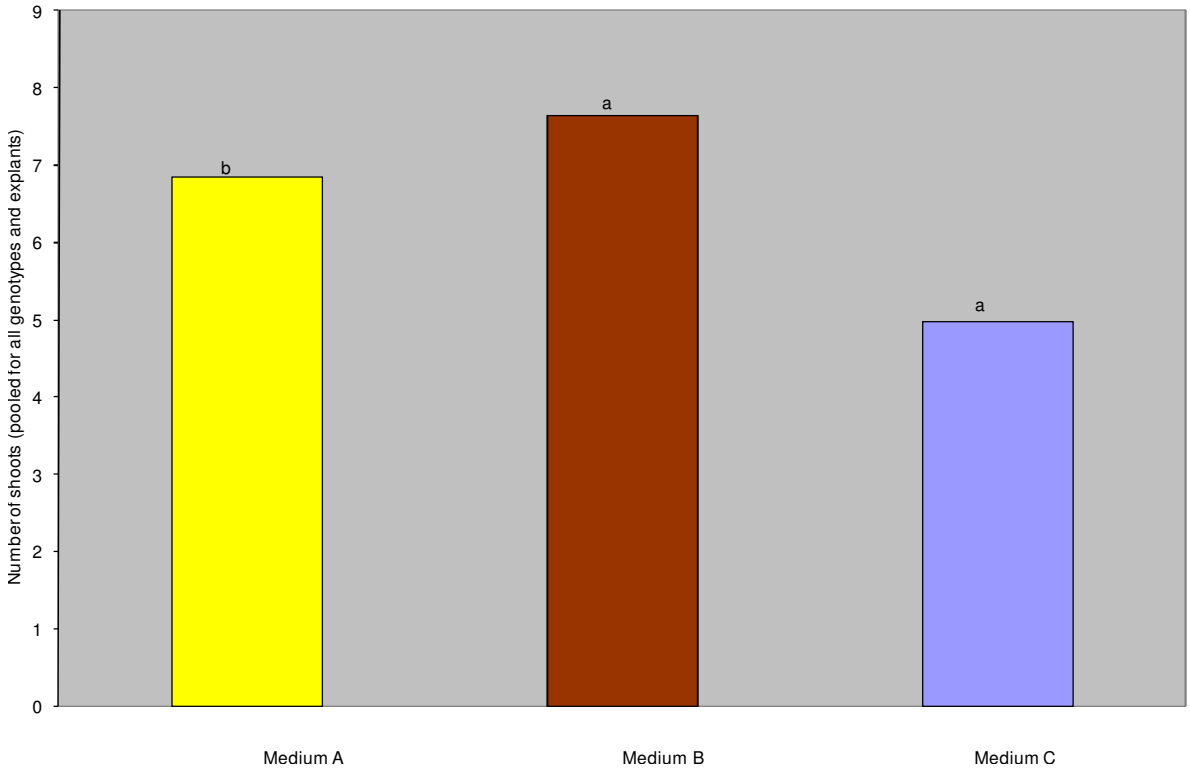

Figure 4. Number of shoots (pooled for seven genotypes and three explants) induced by 1.0 mg/L BAP + 0.1 mg/L IAA (A); 2.0 mg/L BAP + 0.1 mg/L IAA (B), and 3.0 mg/L BAP + 0.1 mg/L IAA(C) media scored after 4 weeks of culture. Bars with different letters represent significantly different means ($p<0.01$) using Fischer's LSD procedure.

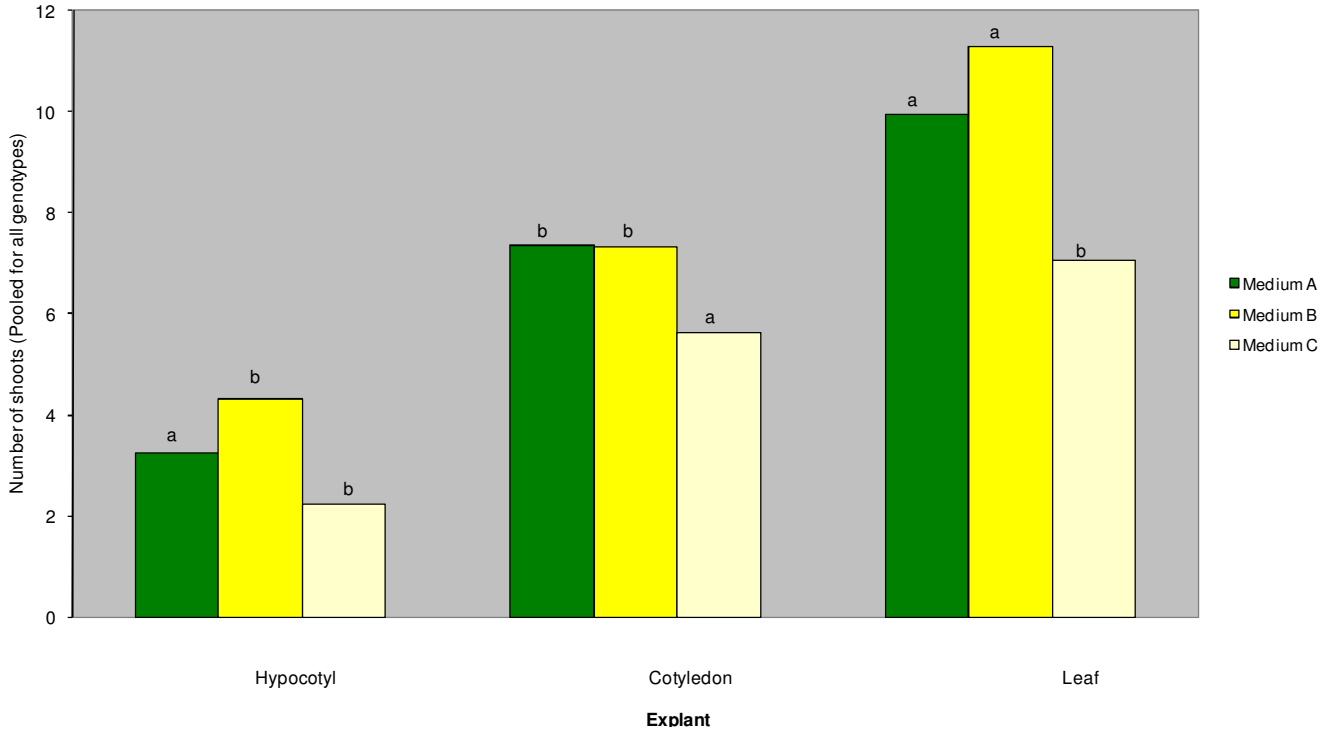

Figure 5. Number of shoots (pooled for five genotypes) induced by 1.0 mg/L BAP + 0.1 mg/L IAA (A); 2.0 mg/L BAP + 0.1 mg/L IAA (B), and 3.0 mg/L BAP + 0.1 mg/L IAA(C) media in hypocotyl, cotyledon and leaf explants after 4 weeks of culture. Bars with different letters represent significantly different means ($p<0.01$) using Fischer's LSD procedure.

tissue cultures were normal diploids (2n = 2x = 24) and without any mitotic aberrations. Similar results were observed in recovery of multiple shoots also from shoot tip explants by Novak and Maskova (1979) in tomato and reported no abnormalities in chromosomes of root tips or no irregularities at meiosis, fruit set, or in seed variability. Thus the present investigation showed that leaf and cotyledon explants were found to be better for regeneration, which is a prerequisite for *Agrobacterium* mediated genetic transformation for the genetic improvement of these important cultivars.

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