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GENOTYPE DEPENDENT DIRECT AND INDIRECT *IN VITRO* REGENERATION IN ELITE TOMATO CULTIVARS

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ABSTRACT

Different tomato cultivars were explored for direct and indirect regeneration response *in vitro*. Combined effect of NAA and BAP was found better for shoot induction (73%) in cv. Nagina compared with KIN and IAA with 58% in Single Node Cutting as explants in cv. Moneymaker. Cotyledon explants proved better for callogenic response (38-45%) in cv. Moneymaker compared with Hypocotyl explants. These findings confirmed that both direct and indirect shoot proliferation in tomato is highly dependent upon genotype, explants and growth regulators.

Keywords: Hypocotyl; Cotyledon; BAP; NAA; Tomato

INTRODUCTION

Tomato is one of the most important vegetable crops grown round the globe in tropical, sub-tropical and temperate areas (Atherton and Rudich, 1986). It is tender perennial, universally cultivated as an annual. In Pakistan tomatoes are grown on an area of 54 thousand hectares with annual production of 540 thousand tones (FAO, 2010;www.faostat.org). Looking at per hectare yield, there is huge yield gap with global tomato leaders as yield in Pakistan is merely 10 tons/ha while it is 17 tons/ha in India and 23 tons/ha in China. This yield gap is attributed to a number of factors such as non-availability of quality seed and cultivars, biotic and abiotic stresses. More than 200 pathogens are reported to reduce tomato production including fungi, nematodes, bacteria and different viruses and viroids (Jones *et al*., 1997; Sarker *et al.*, 2009). Better quality open pollinated and hybrid seeds are normally quite expensive enhancing cost of crop production. Tissue culture offers the mass production of disease/virus free plants of many crops and help in rapid propagation of selected plants with desirable characteristics in shortest possible time. The development of an efficient micro propagation protocol will also be helpful in cost effective multiplication of hybrid plants. Different explants have been explored for direct (Khan *et al*., 2006) and indirect *in vitro* regeneration on different media and plant growth regulators in different combinations (Jatoi *et al*., 2001; Chaudhry *et al*., 2007; Chaudhry *et al*., 2010). Auxin and cytokinin combinations have been more effective for tomato callus induction and regeneration like BAP, KIN and IAA (Chen *et al*., 1999). In the present study we have explored role of NAA, BAP, KIN and IAA combinations to observe regeneration response in different explants in leading tomato cultivars.

MATERIALS AND METHODS

Plant Material and Explant Source: The seed of cultivars Roma, Nagina and Moneymaker were taken from Ayub Agriculture Research Institute (AARI) Jhang road, Faisalabad to raise *in vitro* seedlings on Murashige and Skoog (1962) medium following Khan *et al*., (2006) seed sterilization procedure. Hypocotyl (Hyp; 3-4mm) and Cotyledon explants (Cot; 3-5mm) were excised from 2-3 weeks old *in vitro* raised seedlings and cultured on both M1 and M2 media for direct and indirect shoot induction under light conditions (Table 1). The shoot tip explants were excised with sharp blades and cultured on MS medium in glass jars for further plant multiplication. The developed plants in jars were taken as source for single node cutting (SNC; 8-10mm) and shoot tip (ST; 3-5mm) explants. After inoculation the cultures were placed in the growth room maintained at 25°C ± 1 with fluorescent light intensity of (50 µmole$m^{-2}s^{-1}$).

RESULTS AND DISCUSSION

1. Genotypic response of tomato cultivars for shoot induction (%)

a. NAA and BAP

Among genotypes explored cultivar Roma gave better shoot induction percentage (40-60%) compared with Moneymaker (30-50%) and Nagina (35-55%). In cultivars Roma and Moneymaker the SNC explant type proved

better for shoot induction compared with ST whereas in cultivar Nagina ST explant was better for shoot induction. Among PGRs, the reciprocal combinations of NAA + BAP (2.0 and 1.0 mgL^{-1}; 1.0 and 2.0 mgL^{-1} each) proved better for shoot induction in all the three cultivars. However, maximum shoot induction (73%) was found in SNC explants of Nagina cultivar at NAA + BAP (1.0 and 2.0 mgL^{-1} each) as shown in Fig. 1.

Table 1. Media formulations for direct and indirect shoot induction in tomato cultivars.

Treatments	M1		M2	
	NAA	BAP	KIN	IAA
To	Control	Control	Control	Control
T1	1.0	-	1.0	
T2	2.0	-	1.0	0.1
T3	3.0	-	2.0	0.1
T4	1.0	1.0	3.0	0.1
T5	2.0	2.0	-	-
T6	3.0	3.0	-	-
T7	2.0	1.0	-	-
T8	1.0	2.0	-	-

Table 2. Genotypic response for callus induction on different PGRs in tomato cultivars.

Auxin-Cytokinin Treatments	Explant Types	Cultivars			LSD values
		Roma	Moneymaker	Nagina	
KIN + IAA	Cot	42.07 b	45.53 a	33.93 c	1.04
	Hyp	41.20 a	38.13 b	29.53 c	1.05
BAP + NAA	Cot	58.52 b	62.52 a	33.55 c	0.87
	Hyp	39.15 b	46.11 a	46.78 a	0.72

*Means sharing the same letters in rows are statistically non-significant

b. KIN and IAA

Among tomato genotypes, cultivar Moneymaker proved better for shoot induction (35-58%) followed by Nagina (32-50%) and Roma (28-38%). Among explant types no significant difference was observed in all the cultivars for shoot induction percentage. Higher level of KIN and low auxin IAA (3.0 + 1.0 mgL^{-1} each) proved better for higher shoot induction across genotypes. Overall, cytokinin-auxin combination of NAA and BAP proved better, inducing more shoots compared with KIN and IAA.

2. Genotypic response of tomato cultivars for callus induction (%)

a. NAA and BAP

Among different genotypes explored for callus induction cv. Moneymaker proved better for callus induction (46-62%) compared with Roma (39-58%) and Nagina (46-35%) in Hyp and Cot explant types respectively (Table 1).

b. KIN and IAA

Similar trend was observed for callus induction on KIN and IAA as cv. Moneymaker gave better callus induction (38-45%) compared with Roma (41-42%) and Nagina (29-33) in Hyp and Cot explants respectively (Table 1). Conclusively, Cot explants were found better compared with Hyp for callus induction in cultivar Moneymaker suggesting Moneymaker as the best cultivar for *in vitro* callus induction compared with other genotypes.

Success in tomato regeneration is mainly genotype, explants and PGR or media dependent (Bhatia *et al*., 2004; Jabeen *et al*., 2005). Cytokinin and auxin combinations are reported to induce shoot regeneration in tomato with varying explants source. Our studies reported BAP and NAA as the best cyto-auxin combination compared with KIN and IAA for better shoot induction percentage. These findings are contrary to Jatoi *et al*., (2001) who found BAP and IAA best for callus induction and KIN and IAA for regeneration from ST explants. We also used ST explant however results of Jatoi et al., 2001 could not be verified and the differences may be attributed to genotypic

variation in both experiments. In another report (Gubis *et al*., 2004), they have found zeatin and IAA as the best combination for shoot regeneration from hypocotyl explants. Chaudhry *et al*., (2004) reported combination of IAA, BAP and KIN as the best for shoot formation and hypocotyl as the best explant for maximum callus induction and regeneration (Jabeen et al., 2005). Mohamad *et al*., (2010) reported dominating role of BAP without auxin combination and referred as the best treatment for shoot regeneration in tomato cvs. Pearl and Beril and found Hyp as better explant. Osman *et al*., (2010) reported shoot formation only on TDZ while on NAA and BAP alone and in combinations there was no shoot formation rather these PGRs induced callus in the explants (Batau *et al*., 2002; Chandel and Katiyar, 2000). Osman *et al*., (2010) further advocated the superiority of Hyp explants over Cot explants for callus induction on BAP and NAA containing media. Afroz *et al*., (2009) reported better callus induction from Hyp explants on BAP and IAA in Indian tomato cultivars Avinash, Pusa Ruby and Pant Bahar, however addition of GA_3 in the media enhanced regenerable callus induction. Khan *et al*. (2006) reported profuse embryogenesis all over leaf surface on NAA, NAA and BAP combinations and subsequent regeneration. These findings are contrary to our results as we have observed embryogenic calli with little regeneration potential in Hyp and Cot explants. We have used different explants of the same cultivar and PGRs with different levels and combinations and report differential regeneration frequency.

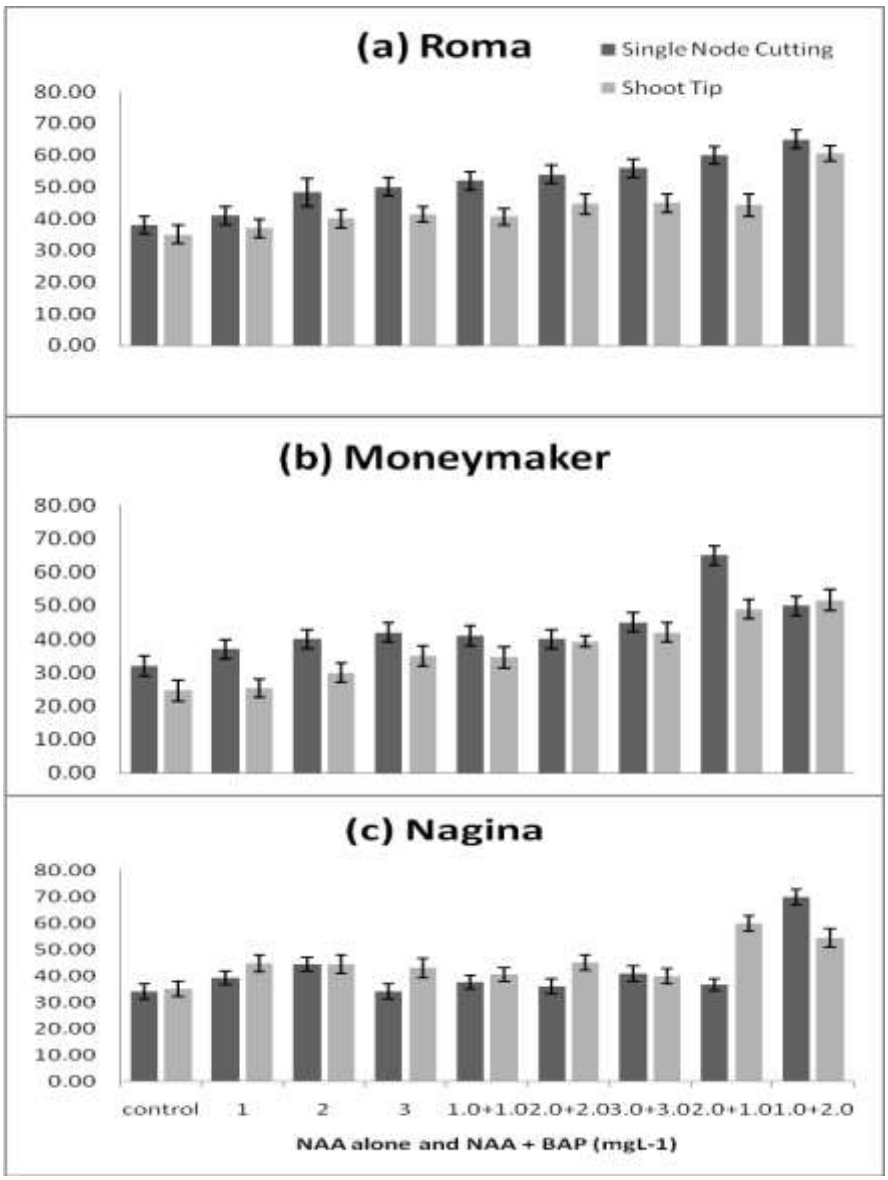

Fig. 1. Genotypic response for shoot induction on NAA alone and NAA + BAP (mgL^{-1}) plant growth regulators in tomato cultivars. Error bars show the standard error values.

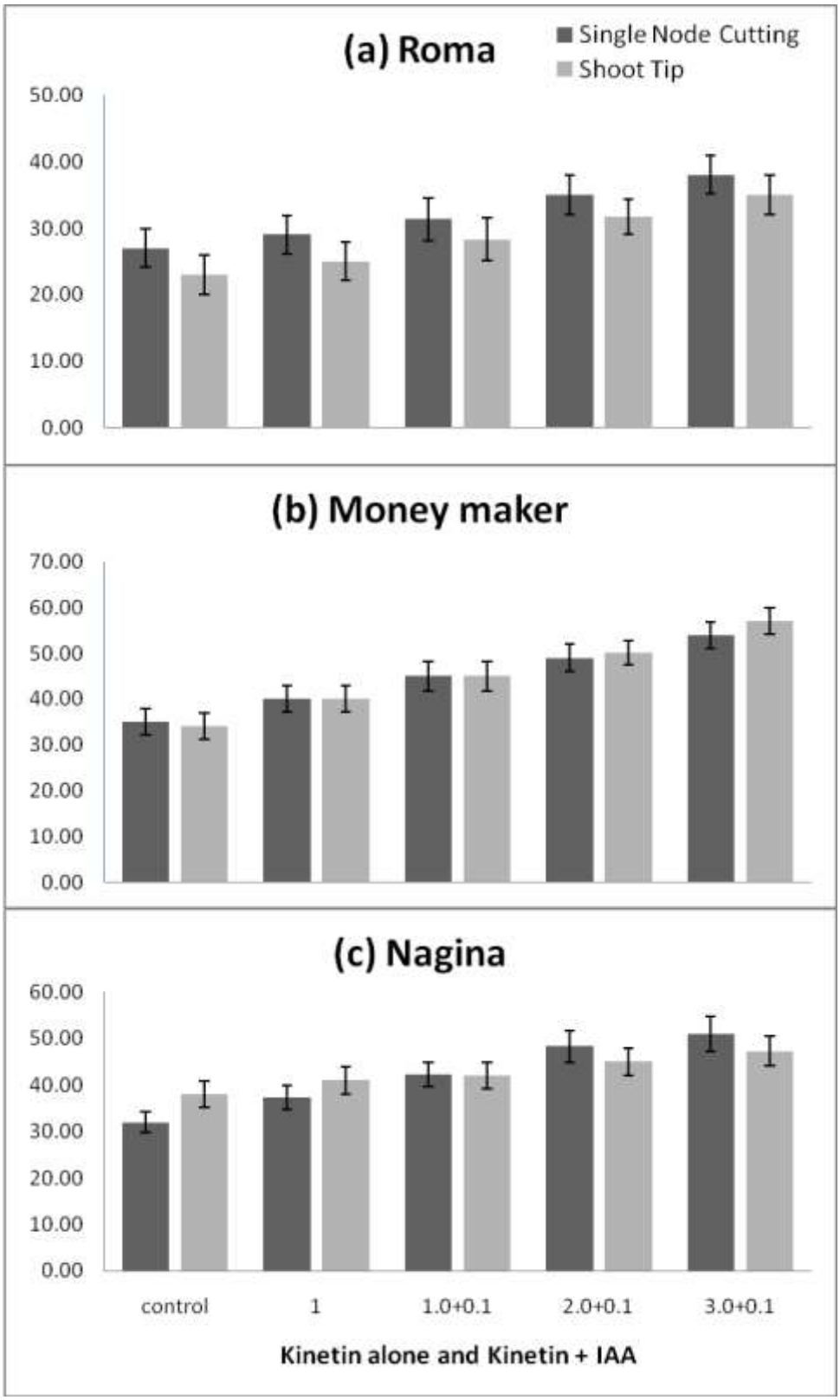

Fig. 2. Genotypic response for shoot induction on Kinetin alone and Kinetin + IAA (mgL^{-1}) plant growth regulators in tomato cultivars. Error bars show the standard error values.

Generally higher cytokinin and low auxin concentrations are promising for shoot induction directly or indirectly. This morphogenic response of the explants is genotype and PGR dependent. We have observed both shoot induction and callus formation with varying frequency in the tomato genotypes Roma, Moneymaker and Nagina. Conclusively, NAA and BAP proved better for both direct shoot induction in SNC explants in cv. Roma and indirect shoot induction in Cot explant in cv. Moneymaker compared with KIN and IAA combinations.

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