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Differential *in vitro* Response of Tomato Hybrids Against a Multitude of Hormonal Regimes

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Abstract: A series of experiments to explore genotypic behaviour of the two F_1 tomato hybrids against a multitude of hormonal regimes were conducted. The diverse explant sources like shoot apices, nodal segments and root sections were subjected to MS media containing different kinds and concentrations of plant growth regulators. Callus induction from cut surface of the shoot tips was more on BAP-IAA enriched media than Kin-IAA enriched media in both hybrids. Both hybrids regenerated 3-7 shoots per culture at Kin-IAA enriched MS media whereas it was 2-3 shoots per culture at BAP-IAA enriched media. A minor callus induction from nodal explants was observed in Kin-IAA enriched media in hybrid Bornia whereas Royesta failed to induce callus. Bornia regenerated 1-2 shoots/culture on Kin-IAA enriched media where as Royesta induced 1 shoot/culture on both media tested. Both hybrids produced non-embryogenic calli from root segments at 100% frequency on MS containing IAA at all levels tested whereas they exhibited a low callus induction frequency on 2,4-D enriched media. Clusters of long roots were found on IAA enriched MS media while no such response was observed on 2,4-D enriched MS media.

Key words: Tomato, *Lycopersicon esculentum*, callogenesis, regeneration, plant growth regulators, *In-vitro* study

Introduction

Tomato is an important Solanaceous crop grown on a wide range throughout the world. It is amenable to physiological and cytogenetic investigation due to its ease of culture and genetic uniformity resulting from autogamy (Rick, 1980). Significant advances have been made during the past decades in the development of *in vitro* culture techniques which have been extensively applied to more than 1000 different crop species (Bigot, 1987). Tissue culture is of great importance for the collection, multiplication and storage of germplasm (Engelman, 1991). Poleschuk and Gorbatenko (1995) observed stimulated growth by exposing tomato shoot apices to phenoxane in culture medium and obtained increased apex-derived culture weight than control. Mirghis *et al.* (1995) was able to get callus formation with a profuse growth rate using apical meristem. Paranhos *et al.* (1996) regenerated plantlets from nodal segments and recorded better yield as compared to plants produced from seed. The plants obtained from *in vitro* cultures of leaf and stem pieces showed more compact appearance, higher fruit set and better root quality than control plants obtained from seeds (Krzyzanowska *et al.*, 1999).

Previously reported *in vitro* response of leaf (Jatoi *et al.*, 1995), Hypocotyl (Jatoi *et al.*, 1997), and internodal segments (Jatoi *et al.*, 1999) against different hormonal regimes. In the present study we explored the collagenic and regeneration potential of shoot apices, lateral buds/nodal segments and root segments on different hormonal levels.

Materials and Methods

Seeds of F1 hybrids of tomato viz. Bornia and Royesta were kindly provided by the vegetable research programme of the National Agricultural Research Centre, Islamabad. The hybrids were developed and marketed by Royal Sluis of the Netherlands. The hybrids have been tested locally for their suitability to protected cultivation for off season vegetable production (Farooq *et al.*, 1998). Surface sterilization of seeds were accomplished by dipping in 70% ethanol for one second. Secondly, seeds were treated with 0.5% NaOCl solution for 10 minutes. Both steps were followed by thorough rinsing with autoclaved distilled water. The same procedure was also followed for sterilization of nodal segments/lateral buds taken from field grown plants.

Basal medium used in this study was Murashige and Skoog (1962) containing thiamine HCl (0.1 mg/l), myo-inositol (100 mg/l), pyridoxine HCl (0.5 mg/l), nicotinic acid (0.5 mg/l) and sucrose (30 g/l). The seedlings thus germinated served as explant source i.e., shoot tips and root segments. The pH of the medium was adjusted to 5.8 with HCl subsequent to the addition of plant growth regulators (PGRs) but prior to the addition of agar. Basal medium was supplemented with a multitude of hormonal combinations. Aliquots of 5 ml of medium were dispensed into test tubes and autoclaved at 1.1 Kg/cm^2, at 121°C for 15 minutes. Shoot apices and root segments each 1 cm long excised from *in vitro* grown seedlings and nodal segments (1 cm) were excised from field grown plants. These explants were aseptically inoculated into the nutrient medium and incubated at 25+2°C under 16 hrs day length maintained by white florescent lamps. Multitude of hormonal regimes tested in this study include;

Shoot apex:	Kin (0,15,20 μM/l) + IAA (0,6 μM/l) and BAP (0,5,10,20,30,40 μM/l) + IAA (0,0.1 μM/l)
Nodal segments:	Kin (0,15,20 μM/l) + IAA (0, 0.5 μM/l) and BAP (0,15,20,25 μM/l) + IAA (0,0.5 μM/l)
and	
Root segments:	2,4-D (0,8,16,24 μM/l) and IAA (0,8,16,24 μM/l).

Results and Discussion

The diverse explants source (Shoot tips, nodal segments and root segments) of the two tomato hybrids Bornia and Royesta behaved differently to a multitude of hormonal combinations. Callogenesis and regeneration behaviour of the two hybrids was dependant on explant source, genotype and PGR tested.

Shoot tips/apices:

Callogenesis: Approximately 1 cm shoot tips/apices taken from *in vitro* seedlings were cultured on different concentrations of plant growth regulators (PGRs). Both hybrids induced callus from cut surface of the shoot apices on control. As control was devoid of PGRs, probably this callus was induced due to indigenous hormonal activity. Callus induction remained with in a range of 65-90% and a meager quantity of callus was observed with pale green coloration (Table 1 and 2). With the addition of Kin and IAA, callus induction frequency was

Table 1: Collagenosis and regeneration in excised terminal shoot tips in response to different concentrations of Kinetin and indole-3-acetic acid

PGRs (μM/l)				Regeneration	
Kin	IAA	Hybrid	Callus (%)	Shoot (%)	Av. No.
0	0	Bornia	70(+)	70	1
		Royesta	65(+)	65	1
	6	Bornia	90 (+++)	90	7
15		Royesta	100(++)	100	4
	6	Bornia	90(+++)	90	3
20		Royesta	88(++)	88	3

+ = Minor; ++ = Medium ; +++ = Excellent

Table 2: Callogenesis and regeneration in excised terminal shoot tips in response to different concentrations of 6-Benzylaminopurine and Indole-3-acetic acid.

PGRs (μM/l)				Regeneration	
BAP	IAA	Hybrid	Callus (%)	Shoot (%)	Av. No.
0	0	Bornia	80(+)	80	1*
		Royesta	90(+)	90	1
5	0.1	Bornia	100(++)	100	3
		Royesta	63(++)	63	3
10	0.1	Bornia	100(++)	100	2
		Royesta	100(++)	100	3
20	0.1	Bornia	100(++)	100	2
		Royesta	100(++)	100	2
30	0.1	Bornia	57(++)	100	2
		Royesta	83(++)	57	1
40	0.1	Bornia	67(++)	83	2
		Royesta	67(++)	67	1

+ = Minor; ++ = Medium ; +++ = Excellent; * = Poor growth of shoots

Table 3: *In vitro* response of excised lateral buds/nodal segments against different concentrations of Kinetin, 6-Benzylaminopurine and Indole-3 acetic acid`

PGRs (μM/l)					Regeneration	
BAP	BAP	IAA	Hybrid	Callus (%)	Shoot (%)	Av. No.
0	--	0	Bornia	-	70	1*
			Royesta	-	60	1
15	--	0.5	Bornia	13(+)	88	2
			Royesta	-	75	1
20	--	0.5	Bornia	-	100	1
			Royesta	-	100	1
25	--	0.5	Bornia	17(+)	100	2
			Royesta	-	100	1
--	15	0.5	Bornia	-	100	1
			Royesta	-	100	1
--	20	0.5	Bornia	-	100	1
			Royesta	-	100	1
--	25	0.5	Bornia	-	100	1
			Royesta	-	100	1

* = Poor growth of shoots ; + = Minor

Table 4: Callogenesis and rhizogenesis in excised root segments in response to different levels of IAA and 2,4-Dichlorophenoxyacetic acid

IAA (μM/l)	2,4-D (μM/l)	Hybrid	Callus(%)	Rhizogenesis
0	--	Bornia	100(+)	100
		Royesta	100(+)	100
8	--	Bornia	100(++)	100*
		Royesta	100(++)	100
16	--	Bornia	100(++)	100
		Royesta	100(++)	100
24	--	Bornia	100(++)	100
		Royesta	100(++)	100
--	8	Bornia	100(+)	--
		Royesta	66(+)	--
--	16	Bornia	80(+)	--
		Royesta	60(+)	--
--	24	Bornia	58(+)	--
		Royesta	67(+)	--

+ = Minor ++ = Medium * = Nodular swellings

accelerated in both hybrids. However, increase in Kin from 15 to 20 μM/l did not respond positively in case of Bornia while for Royesta it reduced the callus induction percentage (Table 1). A profuse callus growth was observed with light green coloration. Shoot apices, when subjected to BAP-IAA enriched media, an increase in callus induction in both hybrids was found with the increase in BAP from 5, 10, 20 and 30 μM/l. However, at the highest concentration of BAP (40 μM/l), callus induction in both hybrids was declined. The extent of callus recorded was minor to medium with light green coloration (Table 2).

Regeneration: Calli induced on cut surfaces regenerated multiple shoots on the same media. The response of both hybrids at Kin-IAA enriched media was found to be better for shoot regeneration as the two hybrids regenerated 3-7 shoots/culture, with a normal growth of shoots. However, both hybrids regenerated 2-3 shoots per culture at BAP-IAA enriched media. Mostly shoot regeneration was indirect via callus. Kin in association with IAA was found to be suitable for regeneration as compared to BAP-IAA enriched media. This difference could also be attributed to concentrations of IAA which was higher (6 μM/l) in former case (Table 1) while lower (0.1 μM/l) in latter case (Table 2). However, further study is suggested to confirm these findings. No root formation in either hybrids was observed in this study. Schnapp and Preece (1986) observed axillary shoot proliferation from shoot tips on MS medium containing 4 mg/l BAP. In an other study, Izadpanah and Khosh (1992) obtained the higher shoot proliferation using 3 mg/l Kin. Shoot proliferation without any callogenesis from cut surface using Kin and GA3 was obtained by Polevaya (1988).

Nodal segments/lateral buds:

Callogenesis: Nodal segments of both hybrids about 1 cm long, taken from field grown plants were cultured on Kin-IAA and BAP-IAA enriched media, having both BAP and Kin at 0,15,20 and 25μM/l while IAA at 0.5 μM/l (Table 3). A minor callus induction was observed in Kin-IAA enriched media in hybrid Bornia whereas Royesta failed to induce callus on all combinations tested. In case of BAP-IAA enriched media, none of the hybrids induced callus on any of the hormonal regimes tested.

Regeneration/shoot emergence: In both media, none of the hybrids produced/regenerated roots but 1-2 shoots/culture were observed in Bornia on Kin-IAA enriched media and Royesta induced 1 shoot/culture on both media (Table 3). The two shoots per culture in Bornia were observed only on those hormonal combinations that exhibited callogenesis. Poor growth of shoots in both hybrids was recorded. No rooting response was observed in either hybrids. Poor growth of shoots and poor callus induction frequency may be attributed to sterilization of nodal segments (derived from field growth plants) with detergent and other chemicals. Complete plantlets regeneration from nodal segments using Kin-NAA enriched MS media was observed by Paranhos (1996). Deng *et al.* (1988) obtained plantlets in large numbers from adventious buds cultured on MS or MH media with various PGRs.

Root segments:

Callogenesis: Root segments (1 cm long) were cultured on MS medium enriched with either IAA or 2,4-D each at 0, 8, 16 and 24 μM/l. Both hybrids produced calli at 100% frequency when their root segments were cultured on MS media containing IAA at all levels tested. These calli were small in size, slightly brownish and had a soft appearance. Both hybrids exhibited a low callus induction frequency at all levels of 2,4-D tested (Table 4).

Rhizogenesis: Root-derived callus of the two hybrids was non-embryogenic. Clusters of long roots were found on IAA enriched MS media (Table 4). None of the hybrids showed any rhizogenesis on 2,4-D enriched MS media. Both hybrids exhibited little rhizogenesis on control media devoid of any hormone. Dodds and Roberts (1982) also obtained extensive root culture without any growth regulator. In case of IAA enriched media, some nodular swellings were observed. They were icy in appearance and white in colour. No such response was observed in case of 2,4-D enriched media.

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