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GENOTYPE DEPENDANT CALLOGENIC AND MORPHOGENIC RESPONSE OF LYCOPERSICON ESCULENTUM HYBRIDS

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Leaf explants from field grown F_1 hybrids of *Lycopersicon esculentum*, namely Bornia and Royesta were cultured on MS media containing 5 x 5 diallelic combinations of IAA and Kin at 0, 4, 8, 12 and 16 µM/l each. Callogenesis, root and shoot regeneration potential of these hybrids were compared on these hormonal regimes. Royesta exhibited better callogenesis response (85%) than Bornia (72%). However, callogenesis on a given hormonal regime was genotype dependant. Root and shoot regeneration was better in case of Bornia than in Royesta. Hybrid Bornia produced shoots much more frequently (6-14%) on many diverse hormonal regimes than Royesta which produced shoots only rarely (5%) on a single hormonal regime.

Keywords: Lycopersicon esculentum, Callogenesis, Regeneration, Plant growth regulators

1. Introduction

Tomato is the second most important cash crop among vegetables and is very popular in the world. It is prone to many biotic and abiotic stresses such as herbicide sensitivity, insect attack, post harvest losses, over-ripening, heat and salt sensitivity. A great treasure of tomato genetic resources is available including modern hybrids, old varieties, breeding lines and wild relatives which serve as raw material for genetic improvement of existing tomato cultivars. A number of methodological approaches such as breeding, screening of somaclonal variations, intergeneric protoplast hybridization and gene manipulation are being currently practiced to achieve genetic improvement of tomato crop. Genetic manipulation of tomato for improved characters requires prior optimization of the conditions for *in-vitro* culture initiated from diverse explants.

Tomato plants obtained by culturing shoot and leaf segments have shown to contain highest sugar content and highest ascorbic acid in tomato fruit and have given higher yields than the control plants obtained from seeds [10 & 5]. 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 [9]. Bacterial wilt resistant tomato plants were obtained from cultured leaf and stem segments and are planned to be cultured on mass scale in humid tropics of India [13]. It was possible to produce somaclonal variants for many agronomic traits such as plant height, plant spread and fruit yield. The *in-vitro* regeneration system has been successfully used in creating somaclonal variations in tomato for resistance against TMV-Flavum virus, without using any chemical or radioactive mutagen [1].

Previously, we reported callogenic and morphogenic responses of tomato when hypocotyl, [7], internodal [8] and leaf explants [6] from *in-vitro* grown plants were used. In this study, we used leaf segments excised from tomato plants grown in vivo from seeds of two F_1 hybrids, as explants and tested their ability for callogenesis and morphogenesis.

2. Materials and Methods

2.1. Plant material

Seeds of F_1 hybrids of tomato viz. Bornia and Royesta were generously provided by the Vegetable Research Programme of the National Agricultural Research Centre, Islamabad. The hybrids were developed and marketed by Royal

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Sluis of the Netherlands. The hybrids have been tested locally for their suitability to protected cultivation for off season vegetable production [3]. Seeds were sown in soil in green-house to obtain seedlings which were transplanted in pots containing sand, soil and farm yard manure in equal proportions, for 8 weeks. Whole leaves were surface sterilised with 0.5% sodium hypochlorite (NaOCl) for 10 minutes and rinsed with autoclaved distilled water thrice. Prior to this treatment, leaves were given a dip in 70% ethanol for one or two seconds and then rinsed with sterile distilled water. Leaf explants (5 x 5 mm) were aseptically dissected from thus disinfected leaves to culture them on sterile growth medium.

2.2 Media

Basal medium as outlined by [14] containing thiamine HCl (0.1 mg/l), myoinositol (100 mg/l), pyridoxine HCl (0.5 mg/l), nicotinic acid (0.5 mg/l) and sucrose (30 g/l) was used. It was supplemented with diverse hormonal regimes including IAA and Kin at 0, 4, 8, 12 and 16 μM/l each. All possible combinations were used between 0, 4, 8, 12 and 16 μM/l of each. Media was solidified with 6 g/l agar after adjustment of pH at 5.8 with either KOH or HCl. Media was dispensed into test tubes at 10 ml/l each and autoclaved for 15 minutes at 121°C.

In an other experiment, calli obtained from leaf explants as described above, were transferred onto MS media containing either Benzyle Amino Purine (BAP) (9 μM/l) or Kinetin (Kin) (9.3 μM/l) alongwith Indole Acetic Aid (IAA) (2.8 μM/l) or Indole Butyric Acid (IBA) (2.4 μM/l).

2.3. Culture Conditions

After inoculation, cultures were incubated in growth chamber where temperature was maintained at 25±2°C. A 16:8 h photoperiod of light intensity of 2000 lux was maintained. Cultures were evaluated for their callogenic and morphogenic response after four weeks.

3. Results and Discussion

Leaf discs were excised from 8 weeks old field grown tomato plants and cultured on MS media which contained IAA and Kin each ranging from 0-16 μM in diallel mode and their callogenic and morphogenic responses were recorded.

3.1 Callogenesis

Plain MS media without PGRs failed to support callogenesis of leaf explants of hybrid Bornia but Royesta exhibited 14% callogenesis on the same media. When kinetin concentration was increased from 0, 4, 8, 12 to 16 μM/l while keeping the concentration of IAA at a fixed level, it was noticed that callogenesis response tended, in general, to increase to a maximum at an optimal concentration of kin but subsequently dropped with the further increase in the concentration of kin. Thus, kinetin seems to supplement IAA for induction of callogenesis upto certain optimal level after which it tends to inhibit callogenesis (Fig 1-a to e). On the contrary, if IAA level is raised in the same order while keeping the concentration of kin fixed at a certain level, callogenesis was found to increase linearly with the increasing level of IAA without its inhibitory effect at any level tested in this study (Fig 2-a to e). In case of Royesta, however, the linear relationship between callogenesis response and IAA levels was limited upto 12 μM/l of IAA beyond which IAA had an inhibitory effect on callogenesis of this variety. Maximum frequency of callogenesis in Bornia (72%) was scored at 8μM/l kin and 16 μM/l IAA, while it was 85% in case of Royesta and was recorded at a concentration of 8 μM/l each of IAA and kinetin.

In Royesta hybrid, IAA and kin seem to have a synergistic effect on callogeneis since increasing the level of kin from 0, 4, 8, 12 to 16 μM/l, the inhibitory effect of IAA on callogenesis was extended further to its higher levels. In Bornia, synergism was not observed between the two hormones for callogenesis (Fig 2a-e). In both hybrids, IAA had a positive effect on callus quality, as calli obtained on media containing IAA had a fine texture as compared to calli obtained on media devoid of IAA .

Genotype dependent callogenic response observed in this study corresponds with the findings of [15] who experimented on many genotypes of tomato to compare their morphogenic and callogenic ability on MS media supplemented with IAA and BAP and found differential response among the genotypes tested. Callogenesis was reported [16] from leaf explants using Kin and IAA at 2 mg/l and 0.1-1.0 mg/l, respectively. Similar findings were achieved [11] on BAP (2.5-5 mg/l), IAA (0.5 mg/l) and NAA (0.5 mg/l). In our previous study, leaf explants of these hybrids grown *in vitro*, when cultured on similar media showed much higher callogenic response than in the current experiment. This deviation can be attributed to the fact that leaf explants excised from *in-vitro* grown

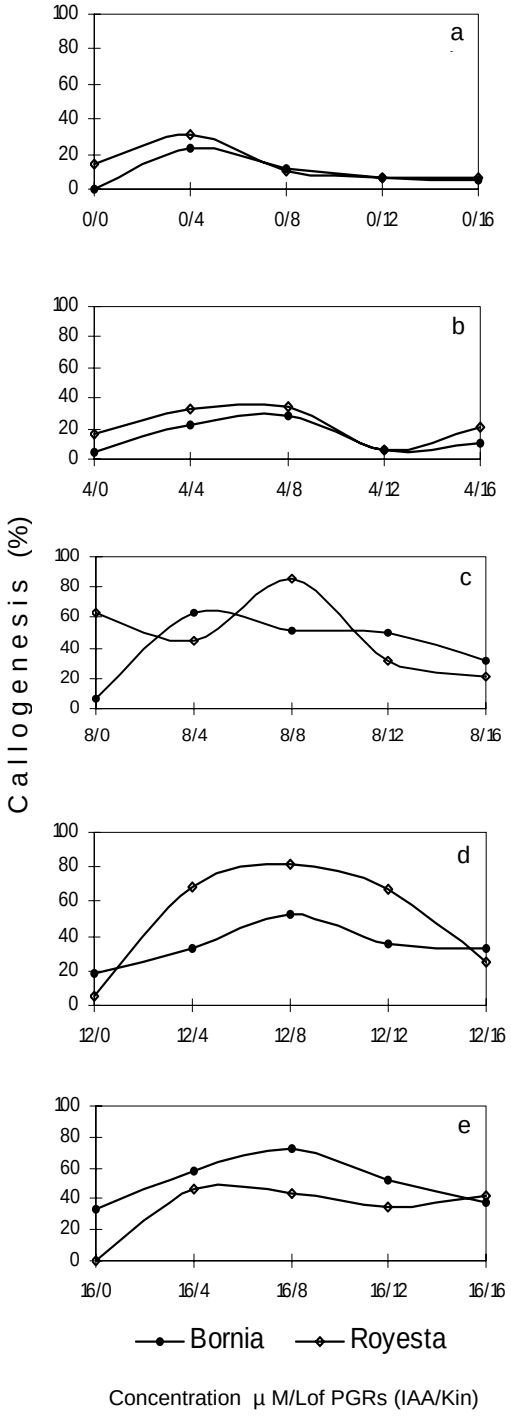

Figure 1(a-e). Callogenesis (%) in response to varying concentrations of IAA alongwith a constant level of Kin.

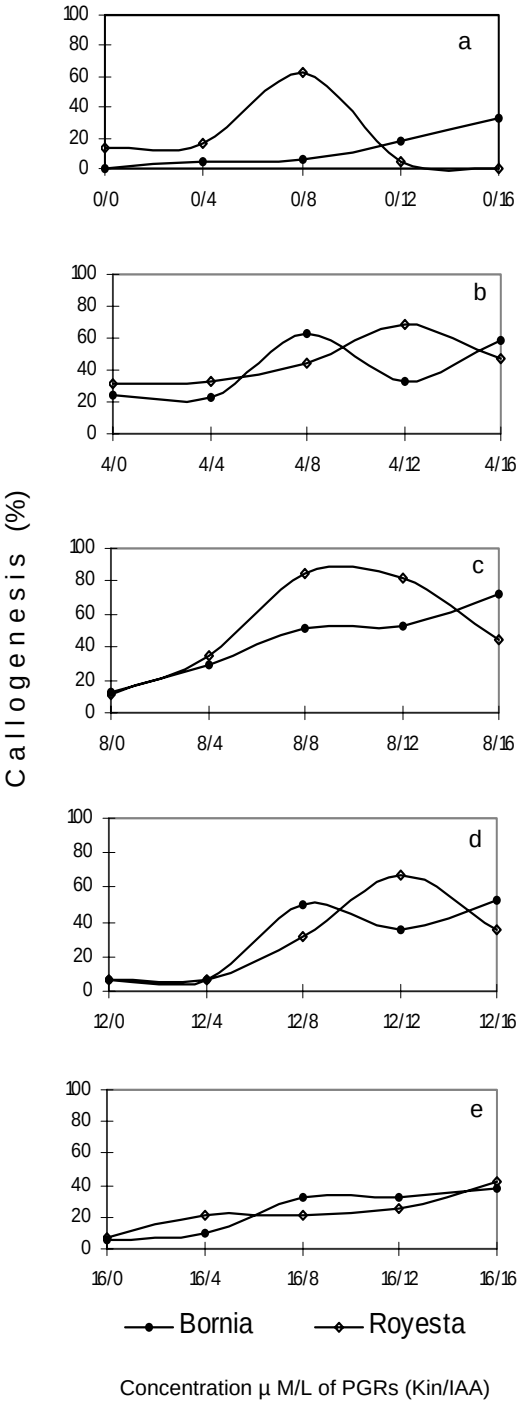

Figure 2 (a-e). Callogenesis (%) in response to varying concentrations of Kin alongwith a constant level of IAA.

Table 1. Regeneration (%) from leaf explants in response to different concentrations of Kin and IAA.

IAA μM/l	Hybrids	Kin (μM/l)							
		0	4	8		12		16	
		Root	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	Bornia	7	-	-	-	-	-	-	-
	Royesta	-	6	-	-	-	6	-	-
4	Bornia	-	-	-	-	-	-	10	5
	Royesta	26	-	-	-	-	-	-	-
8	Bornia	24	5	-	5	-	-	-	-
	Royesta	-	-	-	-	-	-	-	7
12	Bornia	29	6	-	-	14	14	7	7
	Royesta	55	26	-	5	-	8	-	-
16	Bornia	40	11	11	6	-	10	6	6
	Royesta	29	6	-	-	-	-	5	5

plants are exposed to less stringent asepsis treatments than those are from field grown plants.

3.2 Regeneration

Leaf explants developed adventitious roots when cultured on media containing IAA alone. Increasing the IAA levels through 0, 4, 8, 12 to 16 μM/l combined with a simultaneous lower level of kin, seems to enhance the rooting frequencies of leaf discs (Table 1) from 7% to 40% linearly over this range of IAA , in case of Bornia. Royesta, however, exhibited no regular tendency in response of rooting to the PGR regimes tested. Its leaf explants exhibited a maximum rooting frequency of 55% when cultured on 12 μM/l IAA alone.

A very sporadic shoot regeneration was demonstrated by either of the two varieties tested. Bornia turned out to be more responsive to shoot regeneration at many hormonal regimes as compared to Royesta which produced shoots only at 16 μM of kin and IAA each (Table 1 and 2). Genotypic differences in response to IAA and kinetin levels were also reported by [2] who noticed differential morphogenic response of hybrid and non-hybrid tomatoes to hormonal regimes. Similarly, [12] used five different varieties of tomato for regeneration studies and observed genotypic differences in morphogenesis.

In an other experiment, calli obtained from leaf discs cultured as described were transferred to MS media containing either 9 μM/l BAP and 2.8 μM/l IAA; 9.3 μM/l Kin and 2.8 μM/l IAA; 9μM/l BAP and 2.4 μM/l IBA or 9.3 μM/l Kin and 2.4 μM/l IBA. In this experiment, Bornia hybrid demonstrated varying degrees of shoot regeneration responses on all hormonal regimes tested, whereas Royesta showed shoot regeneration only on three of the regimes tested (Table 2). In general, 25-60% calli transferred to these regimes were able to regenerate into shoots depending on the media and the genotype. Rooting frequency of the calli also varied from 20-60% depending on the genotype and the hormonal regime.

The rooted tomato plants obtained from the study may be further investigated for appearance of off-types in the regeneration system developed for the two hybrids tested in these experiments. It is expected that most of the plant obtained in the direct regeneration protocol described in this paper will be true to type as [4] have obtained true to type plants of tomato from leaf and shoot explants cultured on MS media supplemented with plant

Table 2. Regeneration (%) from leaf derived calli in response to different kinds and concentrations of PGRs.

PGRs (μM/L)				Hybrid	Regeneration (%)	
BAP	Kin	IAA	IBA		Shoots	Roots
0	0	0	0	Bornia	-	-
				Royesta	-	-
9	-	2.8	-	Bornia	25	-
				Royesta	20	20
-	9.3	2.8	-	Bornia	60	60
				Royesta	40	-
9	-	-	2.4	Bornia	40	-
				Royesta	-	-
-	9.3	-	2.4	Bornia	53	33
				Royesta	7	7

growth regulators. They were able to regenerate plants via somatic embryogenesis and organogenesis by using 2,4-D, Kin, BAP, NAA and IAA. It is envisaged that the plants obtained through callogenesis mediated regeneration protocol will however show some somaclonal variations requiring further evaluation.

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