

## MANIPULATION OF INTERNODAL SEGMENTS OF F<sub>1</sub> TOMATO HYBRIDS RAISED *IN VITRO* UNDER DIFFERENT REGIMES OF PLANT GROWTH REGULATORS

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### Abstract

The relative morphogenetic response of internodal segments of tomato hybrids viz., Bornia and Royesta was studied at varying concentration of BAP, Kn, NAA and IAA. NAA @ 3  $\mu$ M/l proved to be better than IAA @ 3  $\mu$ M/l in combination with different PGR regimes of both BAP (0, 3, 6, 9  $\mu$ M/l) as well as Kn (0, 6, 12, 18  $\mu$ M/l) exhibiting maximum callus formation in both the hybrids whereas its response for root regeneration was not satisfactory. The influence of IAA in combination with both cytokinins was comparatively less callogenetic but for root regeneration its response was better than NAA. A little shoot regeneration in both hybrids was observed when callus was subjected to regeneration medium containing 2 mg/l and 0.1 mg/l of IBA.

### Introduction

Tissue culture has become a popular technique for asexual propagation of cultivated plants. A variety of cells and tissue of tomato including meristem, hypocotyl, cotyledon, stem section, petiole, leaf and anther have been used to regenerate tomato plants *in vitro* (Young *et al.*, 1987). The impact of genotype on initiation of tissue culture studies and on their organogenic or embryogenic capacity is well documented. Direct shoot regeneration is of pivotal importance in maintaining genetic identity of the micropropagated plants. *Lycopersicon* spp., have been propagated by direct multiple shoot induction at both embryogenic and reproductive stage of the life cycle (Young *et al.*, 1987). Kurtz & Lineberger (1983) have also reported genotypic differences in shoot regeneration capacity of tomato. The present report describes the relative *in vitro* morphogenetic response of stem segments of two F<sub>1</sub> tomato hybrids which suits the off season production under plastic tunnels.

### Materials and Methods

Seeds of tomato F<sub>1</sub> hybrids viz., Bornia and Royesta obtained from Royal Sluis of Netherlands were used. Seeds were surface sterilized by dipping in 70% Ethanol for one second then rinsed with sterile distilled water followed by treatment in 0.5% NaOCl solution for 10 minutes and again rinsed with sterile distilled water.

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Seeds were germinated in test tubes on MS medium (Murashige & Skoog, 1962) containing Myoinositol, Nicotinic acid, Pyridoxine-HCl, Thiamine-HCl, 3% sucrose and 0.6% agar, which after germination furnished as explant source. The pH of the medium was adjusted to 5.8 with HCl subsequent to the addition of phytohormones but prior to the addition of agar. To stimulate proliferation the basal medium was supplemented with different concentrations of BAP and Kn in combination with constant level of NAA and IAA. Aliquots of 5 ml of medium each containing various concentrations of PGRs, were dispensed into Pyrex test tubes and autoclaved at  $1.46 \text{ kg/cm}^2$  for 20 minutes.

Internodal segments (5 mm) of *in vitro* grown seedlings were dissected aseptically. The internode pieces were inoculated onto the nutrient medium and incubated at  $23 \pm 2^\circ\text{C}$  under 16 hrs photoperiod supplied by warm white fluorescent lamps. Each treatment was replicated 12 times. Treatments were evaluated on the basis of callus growth, rooting and shoot regeneration.

## Results and Discussion

Two tomato hybrids viz., Bornia and Royesta were studied for their response to varying concentrations of BAP, Kn, NAA and IAA in a series of experiments (Table 1). Results were mostly dependant on kind and concentration of plant growth regulators and genotype studied.

**Callogenesis:** Callogenesis was observed on all the media combinations where BAP and NAA were used. Maximum callus score (100%) was recorded in both tomato hybrids regardless of increasing concentrations of BAP and genotype. BAP and NAA combination @  $3 \mu\text{M/l}$  each was found optimum for initiating maximum callus in both the hybrids. Such similar observations have been made by Chin *et al.*, (1981).

With increasing concentration of BAP, callogenesis in both hybrids increased upto 100% which indicated a positive response of hybrids towards callogenesis. The comparison of both hybrids displayed a dominating behaviour of Royesta over Bornia for callogenesis as Royesta induced 85, 100 and 100% callus while Bornia 75, 90 and 100% on media supplemented with  $3 \mu\text{M/l}$  IAA and 3, 6 and  $9 \mu\text{M/l}$  BAP. However, media combination with BAP ( $9 \mu\text{M/l}$ ) and IAA ( $3 \mu\text{M/l}$ ) gave the same results with best callogenesis (100%) in both hybrids. Similar results were reported by Kupchinskene (1988) with 2,4-D @ 4-6 mg/l and BAP @ 0.25 mg/l.

In another experiment the effect of different levels of Kn alongwith NAA and IAA on both the hybrids was studied. A 100% callogenesis was recorded on all concentrations of Kn. The size of callus was excellent in Bornia and medium in Royesta. The hormonal combination with Kn and NAA @ 6 and  $3 \mu\text{M/l}$ , respectively seemed optimal for both the hybrids.

Both hybrids behaved different when subjected to media containing Kn and IAA. Callogenesis in Bornia started from 90% which increased to 100% and then declined to 80% with increasing concentrations of Kn with constant level of IAA. In Royesta from 75% callus initiation it declined to 60% and then increased to 100%. A combination of Kn (12) and IAA ( $3 \mu\text{M/l}$ ) for Bornia whereas for Royesta Kn @  $\mu\text{M/l}$  and IAA @ 3

**Table 1. Callogenesis and regeneration in tomato cvs Bornia and Royesta in response to different plant growth regulators.**

PGR ( $\mu\text{M}/\text{l}$ )		Callogenesis (%)		Regeneration (%)	
BAP	NAA	Bornia	Royesta	Bornia	Royesta
00	00	038 +	--	--	63*
03	03	100 +++	100 +++	--	--
06	03	100 +++	100 +++	--	--
09	03	100 +++	100 +++	--	--
BAP	IAA				
03	03	75 +	85 ++	50	62
06	03	90 ++	100 ++	35	35
09	03	100 ++	100 ++	--	04
Kn	NAA				
06	03	100 +++	100 ++	--	25**
12	03	100 +++	100 ++	--	--
18	03	100 +++	100 ++	--	--
Kn	IAA				
06	03	90 +	75 ++	30	55
12	03	100 ++	60 ++	5	8
18	03	80 ++	100 +++	--	--

--No response, \*Few roots, \*\*Thick and long roots

+ Minor callus, ++ Medium callus, +++ Excellent callus

$\mu\text{m}/\text{l}$  was found optimum Callus size in both hybrids was medium to excellent with light green to pale green in colour.

**Regeneration:** Poor regeneration was found in both the hybrids on MS medium containing BAP, IAA and NAA (Table 1). No shoot and root regeneration was found in Bornia at all the concentrations of BAP with NAA, while Royesta produced only few roots. However, BAP and IAA induced only roots in both hybrids on different combinations. Maximum root initiation was found in BAP and IAA, each @ 3  $\mu\text{M}/\text{l}$ , exhibiting 50% and 62% rooting in Bornia and Royesta, respectively. A similar pattern was observed in both the hybrids where an increase in BAP concentrations reduced root regeneration percentage.

In treatment where Kn @ 6  $\mu\text{M}/\text{l}$  and NAA @ 3  $\mu\text{M}/\text{l}$  was used, 25% regeneration of roots was observed in Royesta while no such response was observed in Bornia. The roots thus obtained were thick and long regenerating directly from explant. Kn in combination with IAA induced roots in both the hybrids. Maximum rooting of 30% in

Bornia and 55% in Royesta was found in Kn @ 6  $\mu$ M/l and NAA @ 3  $\mu$ M/l. IAA instead of NAA, with the same concentration of Kn (6  $\mu$ M/l) proved to be better for Royesta as former induced 55% while later 25% roots in the same hybrid.

Callus derived from both hybrid was subjected to regeneration medium containing 2 mg/l Kn and 0.1 mg/l of IBA. Here Bornia regenerated shoots (15%), while Royesta (10%) with an average of 2.2 shoots/culture. Similarly Cassells (1979) regenerated shoots from the internodal derived callus on MS media supplemented with 2 mg/l zeatin and 0.01 mg/l BA.

The results of the present study would suggest that NAA in combination with both BAP and Kn produced more callus than IAA. On the other hand the effect of IAA for regeneration was better than NAA.

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