EFFECTS OF GROWTH REGULATORS, EXPLANT AND GENOTYPE ON SHOOT REGENERATION IN TOMATO (*Lycopersicon esculentum* c.v. Omdurman)

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ABSTRACT

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Development of protocols for *in vitro* regeneration can provide new advances for the production of stress tolerant cultivars. The present investigation was developed at the laboratory of plant tissue culture, Commission for Biotechnology and Genetic Engineering, Khartoum, Sudan, during the period of February 2008 to July 2009. Shoot tip and cotyledon explants isolated from 8-10-day-old seedlings were aseptically cultured on a Murashige Skoog (MS) medium supplemented with various concentrations and combinations of growth regulators under defined environmental conditions. Benzyladenine (BA), kintin (Kin) and 2-isopentenyladenine (2ip) at various concentrations (0.5 – 5.0 mg/l) individually or in combination with naphthaleneacetic acid (NAA; 0.5 – 1.0 mg/l) were tested. . Both explant and growth regulators type and concentrations influenced shoot proliferation. Shoot tip was more efficient than cotyledon explant. Kin proved to be more effective than BA. The best response (2.0 shoots/explant), however was obtained when shoot tip cultured on MS medium supplemented with kin at 4.0mg L-1. The efficiency of both BA and Kin for multiple shoot induction was negatively affected when combined with NAA. Shoots produced *in vitro* were rooted on half-strength agar-solidified MS basal medium or without growth regulator or 0.5 mgl−1 naphthalenacetic acid (NAA) prior to acclimatization and transfer to green house conditions. Regenerated plants grew in soil to maturity and were found to be fertile and morphological true-to-type. This procedure was found to be applicable to other five cultivars of tomato. Thus, it can be advantageously used in combination with conventional breeding techniques for the development of cultivars resistant to biotic and abiotic stresses.

Keywords: tomato, growth regulator, explants, genotype, adventitious shoot, acclimatization

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important Solanaceous vegetable crop grown throughout the world for its versatile uses. It is one of the most important protective foods as it possesses appreciable quantities of vaitamins and minerals and sometime rightly referred to as poor man's orange (Devi *et al*. 2008). In Sudan, tomato is important vegetable crop ranks second to onion among vegetable crops based on cultivated area (Ahmed *et al*. 2001). It is grown throughout the country where irrigation water and arable land are available (Abdelmageed *et al*. 2003). Its production is affected by various stresses such as disease, high temperature, draught, salinity and its vulnerability to frequent insect and pest attacks. Diseases infestations are notorious factors that reduce crop yields and inflate production costs. *Tomato yellow leaf curl virus* (TYLCV) disease and its vector, the whitefly *Bemisia tabaci* (Gennadius), are the major production constraints in the country (Yassin and Nour, 1965). The high temperatures during summer accompanied by low humidity limit the production of tomato to the cooler part of the year and leads to the seasonality of the crop production (Abdalla and Verkerk, 1968). Hence, there is a need to improve this crop using modern biotechnological approaches.

In vitro techniques are important tools for modern plant improvement programs to produce virus free plants (Moghaleb *et al*. 1999), to introduce new traits into selected plants, to multiply elite selections, and to develop suitable cultivars in the minimum time (Taji *et al*. 2002). Used in conjunction with classical breeding methods, an efficient *in vitro* shoot proliferation and regeneration system could accelerate cultivar development programs. The ability to regenerate plants is crucial to the successful application of *in vitro* techniques (Cao and Hammerschlag, 2000). Several researchers have reported about adventitious regeneration in tomato deal with induction of shoots on hypocotyls, apical meristem, cotyledons, stems petioles, leaves, anthers and inflorescences explants (Moghaleb *et al*. 1999, Raziuddin *et al*. 2004, Brichkova *et al*. 2002, Young *et al*, 1987; Branca *et al*.,1990; Compton and Veilleux 1991). However, the improvement of the adventitious shoot regeneration system using tissue culture methods of tomato plants is still important due to the diverse morphogenic potential of different genotypes (Tomsone *et al*. 2004).

In vitro plant regeneration has been found to depend on many factors, of which most important are: genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed 1999). Keeping in view these factors, aim of the present study was to encompass the effects of growth regulator, explant and genotype on *in vitro* regeneration in tomato cultivars grown in Sudan which can be exploited for genetic improvement programs.

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MATERIAL AND METHOD

Plant material

Seeds of tomato cultivars used in this study were obtained from the Agricultural Research Corporation, Shambat, Sudan.

Surface sterilization and seed germination

Seeds were washed under continuously running tap water for 15 minutes then washed by sterile distilled water. Under laminar flow cabinet seeds were disinfected with Clorox (0.5 % free chlorine) at concentration of 15% v/v for 15 mints then rinsed three times with sterile distilled water.

After surface sterilization, ten seeds were directly transferred to culture bottle and incubated for 10 days at 25°C±2 with a 16 h photoperiod. Data on seed germination were recorded after 10 days of inoculation.
Four different basal media namely, full–salt strength MS (Murshige and skoog, 1962) medium, half –salt strength MS medium, Full –salt strength B5 (Gamborg *et al*. 1968) medium and half –salt strength B5 medium, were evaluated for their effects on *in vitro* germination of tomato seed. *In vitro* raised explants (Shoot tip of 1-1.5 cm length and cotyledon of 0.5-1cm length from 10-15 days – old seedling) .

Effects of plant growth regulators and genotypes on multiple shoots Induction

Cytokinine (2ip, BA and KN) with different concentrations (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) were used singly or in combination with and auxin (NAA) to assess the morphogenetic response of the explants. Cultures were incubated for six weeks at 25°C±2 under cool white fluorescent light and 16 photoperiod.

Rooting of in vitro regenerated shoots and plantlet acclimatization

Shoots were excised and cultured on full and half-strength of MS media supplemented with different concentrations (0.0, 0.1, 0.5 and 1.0 mg/L) of IAA, NAA and IBA for root induction. For acclimatization in *vitro* rooted shoots were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 1:1 ratio. Plants were kept under culture room conditions for 7 days then transferred to green house and placed under shade until growth was observed.

Culture condition and data analysis

Results were observed at regular intervals and data were collected from three independent experiments and analyzed by using analysis of variance procedure (ANOVA) on excel computer program. Means were separated by Duncan's multiple range test (DMRT) (Duncan, 1955) and presented as average ± standard error (SE).

RESULT AND DISCUSSION

In order to establish an efficient *in vitro* regeneration protocol for tomato Omdurman cultivar, mature seeds were surface sterilized by Clorox (Sodium hypochlorite 0.5 % free chlorine) at concentration of 15% v/v for 15 mints before *in vitro* ge

rmination. Disinfection of seeds by using Clorox has already been proved to be essential in tomato tissue culture and *in vitro* seed germination (Chaudhry *et al*.2007). Higher germination rate is an important factor for establishing plant tissue culture and be particularly useful when there is a need to submit a uniform set of seedlings to a treatment (Sakhanokho *et al*. 2001). Tomato *in vitro* seed germination was observed after 8-10days in five different basal media evaluated for their effects on seed germination. The highest rate of germination (100%) was obtained by using half-strength MS basal media (Table1). This result is in line with Gubis *et al*. (2003) and Raj *et al*. (2005) who used half strength MS medium for *in vitro* germination of tomato seed.

Table 1. The effects of different basal media on *in vitro* germination of tomato seed

Basal media	Germination (%)
MS	96
1/2 MS	100
B5	75
1/2 B5	65
MB	58

Determination of the most optimal types and concentrations of plant growth regulators as medium constituents is one of the most important aspects of successful micropropagation, among other *in vitro* factors (Ružić and Vujović, 2008). Here in this investigation, the shoot regeneration of tomato cultivar Omdurman has been stimulated *in vitro* from two different types of explants by using various growth regulator used singly or in combinations (Table 2).

Table 2. Effect of cytokinins on shoot induction from shoot tip explant and cotylednary of tomato cultivar Omdurman after 6 weeks of culture

PG R	Con.	Shoot tip			Cotyledonary node		
		Reg. response (%)	Number of shoots/ explant (mean ± SE)	Shoot length / explant (mean ± SE)	Reg. response (%)	Number of shoots/ explant (mean ± SE)	Shoot length /explant (mean ± SE)
BA	0.0	75	1.0± 0.0 e	4.4±0.5 cdef	77	1.0±0.0 e	1.9±0.3 ijkl
	0.1	85	1.2± 0.1 cde	4.5±1. cd	85	1.0±0.0 e	1.0± 0 l
	0.5	85	1.1± 0.09 cde	3.2±0.4 fghi	85	1.0±0 .0 e	1.2±0.1 kl
	1.0	80 (callus)	1.0± 0.0 e	1.4±0.15 jkl	80 (callus)	1.06±0.06 de	1.1± 0 kl
	2.0	80 (callus)	1.0±0.0 e	1.2±0.09 kl	85 (callus)	1.0±0.0 e	1.1± 0 kl
	3.0	80 (callus)	1.0±0.0 e	1.1±0.06 kl	80 (callus)	1.0±0 .0 e	1.1± 0.1 kl
	4.0	75 (callus)	1.0±0.0 e	1.4±0.10 jkl	80 (callus)	1.2±0.1 cde	1.6±0.2 jkl
	5.0	70 (callus)	1.0±0.0 e	1.2±0.08 kl	80 (callus)	1.0±0.0 e	1.2± 0.1 kl
Kin	0.1	100	1.3±0.1 bcd	4.7±0.4 cd	100	1.0±0.0 e	1.6± 0.1 jkl
	0.5	100	1.2±0.1 cde	4.5±0.4 cde	100	1.0±0.0 e	2.6±0.1 hij
	1.0	100	1.3±0.1 bcd	5.4±0.4 bc	100	1.1±0.09 cde	2.7± 0.2 hi
	2.0	100	1.2±0.1 cde	5.6±0.3 abc	100	1.2±0.1 cde	3.5± 0.2 defgh
	3.0	100	1.5±0.1 b	5.5±0.3 abc	100	1.3±0.1 bcd	4.6± 0.3 cd
	4.0	100	2±0.0 a	4.6±0.3 cd	100	1.4±0.13 bc	6.6± 0.3 a
	5.0	100	1.3±0.1 bcd	3.0±0.09 hi	100	1.3±0.1 bcd	4.7± 0.5 cd
2ip	0.1	100	1.2±0.1 cde	3.0±0.3 hi	100	1.2±0.1 cde	5.0± 0.7 bc
	0.5	100	1.2±0.1 cde	5.1±0.3 bc	100	1.0±0.0 e	6.2± 0.6 ab
	1.0	100	1.2±0.1 cde	3.7±0.3defgh	100	1.2±0.1 cde	4.4± 0.3 cdefg
	2.0	100	1.3±0.1 bcd	4.5±0.2 cde	100	1.1±0.09 cde	3.2± 0.2 efgh
	3.0	100	1.2±0.1 cde	3.1±0.3 ghi	100	1.06±0.06 de	2.1±0 ijkl
	4.0	90 (callus)	1.0±0.0 e	2.6±0.2 hij	90 (callus)	1.06±0.06 de	2.4± 0.1 hijk
	5.0	90 (callus)	1.0±0.0 e	2.1±0.2 ijkl	90 (callus)	1.0±0.0 e	1.8± 0.1 ijkl

*Means followed by the same letter within columns are not significantly different at the 5% level of significance using Duncan's multiple range test

Multiple shoots regeneration of tomato cultivar Omdurman was initiated from the cotyledon and shoot tip explants after 3weeks of culture (Figure 1a). The frequency of shoot formation was influenced by both the type and concentration of phytohormones used.

The dose of cytokinin is known to be critical in multiple shoots induction (Abdellatef and Khalafalla, 2007). Therefore, in this study we compared the response of both explants to various concentrations of BA, kin and 2ip. Kin at different concentrations induced more shoots per explant compared to BA and 2ip at the same concentrations (Table 2). Indicating that, kin was more effective than BA and 2ip for multiple shoot production from tomato cotyledon and shoot tip explants. Also our result showed that the shoot tip was more efficient for multiple shoot induction in tomato than cotyledon explant (Table 2). Gubis *et al*. 2004 reported that the frequency of adventitious shoot regeneration differed depending on the type of explants and both the type and concentration of growth regulators added to the regeneration medium. Moreover, Plana *et al*. 2005 reported that, tomato adventitious shoot capacity depend on explant source.

The results showed that at higher concentration of the cytokinin the regeneration percentage and shoot regeneration frequencies were decreased (Table 2). This is mainly, because at higher cytokinin level explants produced excessive callus and failed to improve the efficiency of shoot multiplication. Thiem (2003) reported that callus growth on explant usually interfere with the propagation process.

In order to evaluate the synergistic effect of BA and Kin with NAA for direct shoot regeneration, it was found that, the combination of NAA with BA or Kin negatively affected the multiplication rate of the tomato compared with cytokinine used singly (Table 3).The inhibitory effect of auxin on multiple shoot induction has been demonstrated in numbers of plants. In cotton (Abdellatef and Khalafalla, 2007), faba bean (Khalafalla and Hattori, 2000) and mung bean (Gulati and Jaiwal, 1992) it was reported that the addition of NAA to medium containing cytokinin did not improve shoot multiplication rate.

Table 3. Effect of cytokinins and auxin combinations on shoot induction from shoot tip explant of tomato cultivar Omdurman after 6 weeks of culture

BA	Kin	NAA	Regeneration response %	Number of shoots / explant (mean ± SE)	Shoot length / explant (mean ± SE)
0.5	0.0	0.1	60	1± 0 c	1.6± 0.1 j
1.0	0.0		77	1.06±0.06 bc	4.8± 0.2 def
2.0	0.0		66	1± 0 c	3.6± 0.2 fgh
3.0	0.0		74	1.06±0.06 bc	2.7± 0.3 hi
4.0	0.0		65	1± 0 c	4.2± 0.4 efg
0.5	0.0	0.5	65 (callus)	1± 0 c	5.3± 0.5 cde
1.0	0.0		65 (callus)	1± 0 c	3.1± 0.3 ghi
2.0	0.0		65 (callus)	1± 0 c	2.3± 0.1 hi
3.0	0.0		60 (callus)	1± 0 c	2.4± 0.1 hi
4.0	0.0		60 (callus)	1± 0 c	2.3± 0.1 hi
0.0	0.5	0.1	64	1± 0 c	6.5± 0.47 abc
0.0	1.0		86	1.2±0.1 b	7.2± 0.47 ab
0.0	2.0		60	1± 0 c	5.8± 0.57 bcd
0.0	3.0		95	1.4±0.13 a	7.7± 0.3 a
0.0	4.0		84	1.2± 0.1 b	6.1± 0.7 bcd
0.0	0.5	0.5	77	1.06± 0.06 bc	6.5± 0.5 abc
0.0	1.0		74	1.06± 0.06 bc	5.2± 0.6 cde
0.0	2.0		71	1.06± 0.06 bc	5.4± 0.5 cde
0.0	3.0		63	1± 0 c	5.3± 0.5 cde
0.0	4.0		75	1.06±0.06 bc	5.2± 0.5 cde

*Means followed by the same letter within columns are not significantly different at the 5% level of significance using Duncan's multiple range test

The plant regeneration capacity of this procedure was found to be applicable to five other tomato genotypes (Table 4). The best result (2shoots/explant) for number of multiple shoots per explant was obtained by the cultivar Omdurman. Earlier it was reported that the regeneration response of tomato to plant growth regulators (PGRs) has been to be highly genotype-specific, and as such, the type and concentration suitable for one genotype may not be optimal for others (Frankenberger *et al*. 1981; Kurtz and Lineberger, 1983; Plastira and Perdikaris, 1997; Bhatia *et al*. 2004).

Table 4: Effect of auxins on rooting of *in vitro* derived shoots of *tomato*, cv Umdorman after six weeks of culture

Auxin	Conc.	Full MS			1/2 MS		
		Rooting response (%)	Number of roots / explant (mean ± SE)	Root Length (mean ± SE)	Rooting response (%)	Number of roots / explant (mean ± SE)	Root length (mean ± SE)
IBA	0.0	100	9.3±0.8 def	4.15±0.2 hij	100	12± 0.8 cdef	5.4±0.3 cdef
	0.1	100	7.7±1.2 ef	5.65±0.3bcde	100	6± 0.7 f	7.40±0.2 a
	0.5	100	8.4±1.3 ef	5.9±0.2 bcd	80	7± 0.6 ef	7.35±0.1 a
	1.0	80 (callus)	15±1.5 abcde	5.1±0.2 defg	85 (callus)	7± 0.5 ef	7.15±0.2 a
IAA	0.1	100	10±1.2 def	4.75±0.2 fghi	89	13± 1.6 bcdef	5.5±0.2 cdef
	0.5	100	11±1.8 cdef	5.15±0.2 def	100	21±1.7 ab	6.1±0.2 bc
	1.0	70	16±1.5 abcde	4.7±0.2 fghi	100	21± 0.7 a	6.3±0.2 b
NAA	0.1	80	19±1.6 abc	3.95±0.2 ij	100	20± 1.5 ab	4.95±0.2 efgh
	0.5	78	17±1.7 abcd	3.35±0.1 jk	100	22± 0.9 a	4.3±0.2 ghi
	1.0	70 (callus)	9.6±1 def	2.3±0.1 l	70 (callus)	11± 1.1 cdef	2.85±0.1 kl

*Means followed by the same letter within columns are not significantly different at the 5% level of significance using Duncan's multiple range test

Regenerated shoots were excised and rooted on full and half-strength MS medium without or with different levels of NAA, IAA, or IBA. Our result showed that 100% rooting was obtained in both full and half-strength MS medium without growth regulators (Table 5).

Table 5. Mean number of shoots (±standard error) formed on shoot tip explants of six genotypes of tomato after six weeks of culture on MS medium supplemented with kin at 4.0 mg/l.

Genotype	Regenerating culture %	Number of shoots /explants (mean ± SE)	Shoot length / explants (mean ± SE)
Omdurman	100	2.0 ± 0.1 a	7.9±0.3 a
Jazeira	100	1.1 ± 0.09 c	8.0 ±0.2 a
Baledi	100	1 .0 ± 0.0 c	4.6±0.4 b
Peto 86	100	1.8 ± 0.09 a	8.0±0.3 a
Castle Rock	100	1.5 ± 0.1 b	8.3±0.5 a
Strain -B	100	1.2 ± 0.1 bc	7.5±0.3 a

*Means followed by the same letter within columns are not significantly different at the 5% level of significance using Duncan's multiple range test.

Similarly, Mensuali-Sodi *et al*. 1995 reported that tomato *in vitro* rooting does not require any exogenous plant growth regulators. However, during our study, cultivation on auxin-containing half-strength MS medium resulted in a large number of rooted microshoots (22± 0.9) and longer root (7.4 ± 0.9 cm) than cultivation on an auxin-free medium. These results can be explained by the promotive effect of auxins on root initials, as observed by De Klerk *et al*. (1999). The beneficial effect of using half-strength MS medium for rooting of *in vitro* induced shoots has already been reported for tomato. Devi *et al*. 2008 reported that the best rooting was found to be in half-strength medium supplemented with 0.2mg/l IBA. After well established root (Figure b) plantlets were subjected to acclimatization. Plantlets were removed from rooting medium after four weeks of incubation and transferred to plastic pots containing autoclaved soil and sand at the rate 1:1 covered with glass bottle to maintain humidity and were kept under culture room conditions for three weeks (Figure 1c). After three weeks, glass bottles were removed and transferred to green house and placed under shade until growth was observed. 95% of plants survived and all were morphologically normal (Figure 1d)

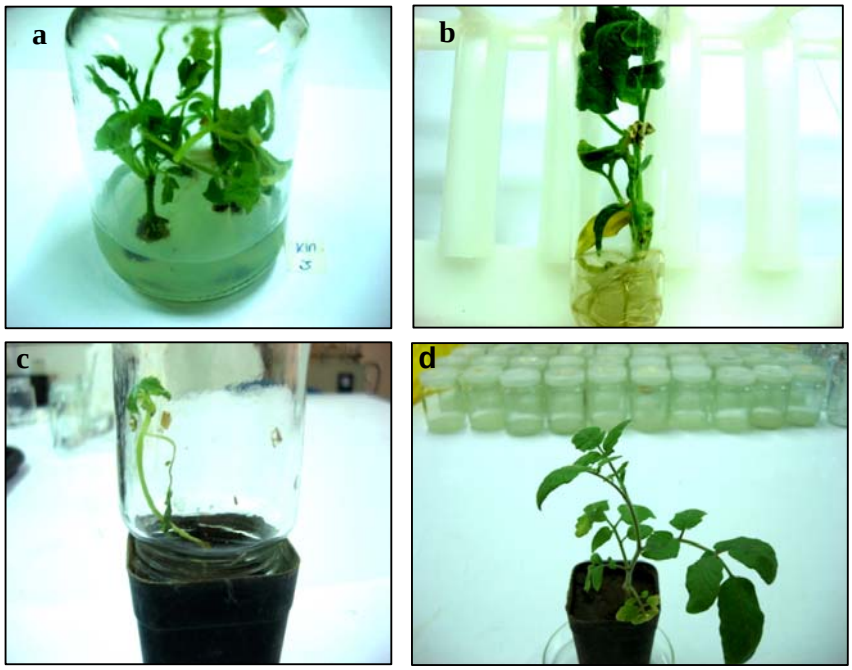

Figure 1. *In vitro* induction of multiple shoots and plant regeneration of tomato cultivar of Omdurman (a) Multiple shoots induced from cotyledonary explants, (b) *In vitro* rooted shoot on half – strength MS basal medium supplemented with IBA (0.1 mg/L), (c) Potting up and hardening of the plantlets, (d) Tomato plant established in soil.

In conclusion development of an efficient tissue culture and plant regeneration protocol for tomato genotypes grown in Sudan is the first step towards the application of modern technology in improving tomato breeding such as using plant tissue culture technology in the area of selection resistance and production of artificial seeds.

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