J. Agric. Sci. Mansoura Univ., 34 (5): 4761 - 4776, 2009

EFFECT OF GENOTYPE, EXPLANT AND KINETIN CONCENTRATIONS ON SHOOT REGENERATION AND EVALUATION OF SALINITY TOLERANCE IN TOMATO

Taha, Sahar S.
Vegetable Crops Department. Faculty of Agriculture, Cairo University

ABSTRACT

In this study, four tomato hybrids (Sarya, Nematoda, Mereto and Abeza)and four wild species (*L. pimpinellifolium* PI344102, *L. peruvianum* CMV-INRA, *L. escu.* PI174263 and *L. escu.* var. *ceriaciforme* PI321749) were used. Hypocotyl and cotyledon explants were isolated from seedling and cultured on modified MS medium (Murashige and Skoog ,1962),which contained MS salts and B5 vitamins (Gamborg *et al.,* 1968), 1% (w/v) agar supplemented with kinetin at levels 0.5 , 1.0 and 2.0 mg/l. The highest percentage of callus was produced in cv. Abeza and *L.pimpin.*PI344102. The highest number of explants that produced shoots was observed in *L. escu.* PI174263 on MS media with 1.0 and 2.0 mg/l KIN. Maximum total number of shoots and number of shoots per explant was produced by culturing cotyledon explants of *L. escu.* PI 174263 on MS media with 2.0 mg/l KIN. Tomato seeds (*L. pimpin.* PI344102, *L. peruv.* CMV-INRA, *L. escu.* PI174263 and *L. escu.* var. *ceriaciforme* PI321749) were cultured on MS medium with 2.0 mg/l KIN and supplemented with different concentrations of sea salt (0.0, 2000, 4000, 6000 and 8000 ppm). The germination percentage and plant fresh weight was the highest in *L.escu. PI*174263. Tallest plants were produced in *L.escu.* PI174263 and *L.escu. var. ceriaciforme* PI3217. Increasing salinity reduced germination percentage, plant height, leaves number and plant fresh weight in all genotypes, except in *L. Pimpin. were* increasing the salinity upto 4000 ppm increased plant height.

Abbreviations: KIN- kinetin; *L.pimpin.* - *L. pimpinellifolium* PI344102; *L.peruv.*- *L. peruvianum* CMV-INRA; *L.escu.* PI174263- *L. esculentum* PI174263 and *L. escu. var. ceri.*- *L. esculentem* var. *ceriaciforme* PI321749.

Keywords: Tomato, Organogenesis, Regeneration, Explant, Media and Salinity.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the second most popular vegetable crop next to potato in the world. Tomato is mostly grown from hybrid seeds , which are expensive due to involvement of manual labor for emasculation and pollination. An efficient tissue culture system may produce hybrid plantlets at low cost. As tomato is grown world – wide , including in marginal and sub marginal lands, a good regeneration system may aid in genetic engineering techniques to develop genotypes resistant to various stresses .The majority of research tests few species of tomato for their ability to produce callus and shoots (Costa *et al.*, 2000 a, b and Venkatachalam *et al* ., 2000). Since the genotypes differ markedly in their response (Stommel and Sinden ,1991 and El – Farash *et al.*, 1992) it is important to test a wide range of genotypes to develop a universally applicable protocol for shoot regeneration in tomato. Among *Lycopersicon* species, *L. peruv.* is considered highly organogenetic and regeneration of

shoots has already been documented (koornneef *et al.*, 1993). Other genotypes were also described by their ability to from shoots from hypocotyls in *L.pimpin.* WV 700 (Faria and Illg,1996), cotyledons in *L. escu.* cv. UC82B (Hamza and Chupeau ,1993) , suspension cells in *L. escu.* cv . VFNT (Meredith, 1979) and protoplasts in *L. escu.* cv. Lukullus (Morgan and Cocking , 1982). The regeneration response of tomato to plant growth regulators has been observed 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* ., 1981a; Kurtz and Lineberger , 1983 ; Plastira and Perdikaris , 1997 and Bhatia, 2004).

The excess of salt in the soil or in the irrigation water is one of the biggest problems in agriculture since almost all cultivated plants are sensitive to it. According to Epstein (1976), salinity is not only a problem in arid and semi-arid regions, but it also occurs in fertile and productive soils where overexploitation of water reservoirs, lack of rain, and use of large amounts of fertilizers caused salt accumulation. Thus, selection of salt tolerant lines is one of the most important challenges in plant biology. One of the problems that appears when evaluating tolerance to a complex stress such as salinity, is the labor intensive process required to screen thousands of plants and the lack of reliable salt stress marks (Cruz *et al.*, 1990; Saranga *et al.*, 1993 and Cano *et al.*, 1996). These difficulties have been the cause that, in certain species such as tomato, few practical results have been obtained from tradltional breeding programs. *In vitro* plant tissue culture has been proposed as a useful, quick and economical tool to evaluate salt tolerance. Although a lack of concordance between growth of callus under salt stress and growth at the whole plant level has been observed in several species (Tal, 1984; McCoy, 1987), in plants such as tomato, positive correlations have been found (Tal *et al.*, 1978; Perez -Alfocea *et al.*, 1994 and Cano *et al.*, 1996). However, use of *in vitro* culture presents numerous disadvantages, such as somaclonal variation, culture medium and explant source effects (Garcia – Reina *et al.*, 1988) and mainly the lack of the whole plant integrity that exclude crucial mechanisms of salt resistance like ion exclusion. To avoid these problems, and as an alternative to the callus growth approach, several authors have evaluated the *in vitro* culture of shoot apices or buds under salinity conditions (Martinez *et al.*, 1996 and Cano *et al.*, 1998). A relatively high salt tolerance was found in some wild types *Lycopersicon* species namly, *L.cheesmanii*, *L.pennellii*, *L.peruvianum* (Saranga *et al.*,1993). Compared to cultivated tomato, its wild counterparts such as *L. pimpinellifolium*, *L. peruvianum* and *L. glandulosum* show better regeneration capabilities (Lech *et al.*, 1996).

The aim of the present work was to study the factors affecting on shoot organogenesis of eight tomato genotypes. Thus, selecting the most appropriate genotype that could use as tolerant rootstock for salinity.

MATERIALS AND METHODS

This investigation was carried out in the Tissue Culture Laboratory of the Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period from 2007 to 2008

Experiment 1. Effect of genotypes, explants and kinetin concentration on shoot regeneration response.

In this study four commercial tomato hybrids and four wild tomato accessions were used (Table, 1). Seeds of all genotypes were surface sterilized by dipping in 70% ethanol for one min. , followed by immersion in 20% sodium hypochlorite for 15 min., and were rinsed three times with sterile water .The sterilized seeds were germinated in jars containing solid MS free hormone media and incubated at 25°C under a 16/8-h light/ dark photoperiod. One week old seedling was used as source of hypocotyl and cotyledonary leaves. Both types of explants were isolated and cultured in jars with modified MS medium, which contained MS salts, 3% (w/v) sucrose, B5 vitamins (Gamborg *et al.*, 1968), 1% (w/v) agar and supplemented with KIN at different concentration (0.5 , 1.0 and 2.0 mg/l) , pH 5.8. The explants were subcultured weekly on corresponding medium freshly prepared for five weeks.

Table 1. Tomato genotypes used in this study and their sources.

Genotypes	Type	Seed supplier
Sarya	Hybrid	Petoseed Co. Ltd
Nematoda	Hybrid	Petoseed Co. Ltd
Mereto	Hybrid	Technogreen Co. Ltd
Abeza	Hybrid	Technogreen Co. Ltd
L. peruvianum CMV-INRA	Wild	Dr.H. Laterrot (INRA, France)
L. pimpinellifolium PI344102	Wild	The U.S.D.A through Dr. Charles Block (Plant Introduction Station, Anes, Iowa)
L. escu. PI174263	Wild	
L. escu. var. *ceriaciforme* PI321749	Wild	

Acclimatization was achieved by transferring shoots 2 - 2.5 mm in length to half strength MS medium. After two weeks, the plantlets which showed a well developed root system were transferred to sterilized vermiculite in plastic cups and irrigated with 1/4 MS solution. After acclimatization for three weeks, the plants were grown under green house conditions.

Experiment 2. Effect of salinity on *in vitro* growth and shoot regeneration of tomato seeds.

The effect of sea salts of different concentrations (0, 2000, 4000, 6000 and 8000 ppm) on growth and shoot regeneration from seeds of the *lyceopersicon* wild species (Table, 1) was tested. Sterilized seeds were cultured in jars containing 30 ml of MS medium with 2.0 mg/l KIN supplemented with different concentrations of sea salt for five weeks. The jars of each experiment were incubated at 25°C under a 16/8-h light / dark photoperiod and were placed in a controlled environment room according to a completely randomized design, with three replications per treatment. Every

replicate contained 20 explants, shoot regeneration and callus formation were observed. Data were subjected to analysis of variance as described by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Experiment: 1

Two explant types derived from cotyledonary leaf and hypocotyl were isolated from eight genotypes of tomato (Table 1). Sixty segment from each type of explants were cultured on MS media supplemented with KIN at different concentrations. Two weeks after the beginning of the experiment, white green and friable calli were obtained at the cut end of the cotyledonary leaf and hypocotyl. One week later shoot developed directly from the explant.

Callus and shoots percentage

Data presented in Table 2 indicates that, regeneration was achieved in all genotypes, there were differences among cvs. on the percentage of explants that produced callus and shoots. The highest percentage of callus was produced in cv. Abeza and *L. pimpin.* PI344102. While the highest percentage of shoots was produced in *L. escu.* PI174263. Direct shoots formation occurred on MS medium with 1 mg/l KIN, while the highest callus percentage was accured on MS medium with 0.5 mg/l KIN. Percentages of explants with calli were high by culturing cotyledon explants, while the percentage of explants with shoots was high by culturing hypocotyl explants. The results of three ways interaction (genotype x explants x medium) revealed that the maximum shoot percentage were formed from cotyledon and hypocotyl explants on MS medium having 1 mg/l KIN of cvs. Nematoda and *L. escu.* PI174263.

Total number of shoots and number of shoots per explant.

Data presented in Table 3 and Fig.1 indicated that higher number of shoots and number of shoots per explant were produced in genotype *L. escu.*PI174263. Insignificant variation was found between different explants. The medium containing 2.0 mg/l KIN induced higher number of shoots and number of shoots per explant. The interaction between genotypes and explants was significantly observed for total number of shoots except in cvs. Nematoda, *L. peruv., L. escu.* PI 174263 and *L. escu.* var. *ceriaciforme.* Insignificant interaction between genotypes and explants for the number of shoots produced per explant except in cvs. Sarya, Abeza and *L. pimpin.* A significant effect of the interaction between genotypes and media was also observed for total number of shoots for all genotypes under study. The maximum number of shoots per explant were produced by culturing the explants in MS medium with 2.0mg/l KIN except in cv. Sarya. The maximum number of shoots per explant was produced by culturing the explants in MS medium with 1.0 and 2.0 mg/l KIN. The results of the interaction between genotypes, explants and media revealed that maximum total number of shoots and number of shoots per explant was produced by culturing cotyledon explant of *L. escu.* PI 174263 on MS medium with 2.0 mg/l KIN.

Table 2. Effect of genotypes, explants and kinetin concentrations on callus and shoot percentage (after five weeks) in tomato.

Genotypes	Explant	Callus %				Shoots %			
		0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN	Mean	0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN	Mean
Sarya Hybrid	Cotyledon	100.0	6.12	72.57	59.62	0.01	93.88	27.25	40.38
	Hypocotyl	17.89	0.00	27.61	15.17	82.11	100.0	72.64	84.92
Mean		58.94	3.07	50.18	37.40	41.06	96.94	49.95	62.65
Nematoda Hybrid	Cotyledon	46.67	0.00	0.00	15.56	53.33	100.0	100.0	84.44
	Hypocotyl	39.86	0.00	10.53	16.80	60.14	100.0	89.47	83.20
Mean		43.26	0.00	5.27	16.18	56.74	100.0	94.74	83.82
Mereto Hybrid	Cotyledon	0.00	0.00	0.00	0.00	100.0	100.0	100.0	100.0
	Hypocotyl	74.70	6.94	26.35	36.00	25.30	93.06	73.65	64.00
Mean		37.35	3.48	13.18	18.00	62.65	96.53	86.82	82.00
Abeza Hybrid	Cotyledon	100.0	11.66	0.00	37.22	0.01	88.34	100.0	62.78
	Hypocotyl	100.0	100.0	53.15	84.38	0.01	0.01	46.85	15.62
Mean		100.0	55.83	26.58	60.80	0.01	44.18	73.43	39.20
L. pimpinellifolium PI344102	Cotyledon	100.0	100.0	92.11	96.37	0.01	0.01	7.77	2.60
	Hypocotyl	12.22	0.00	0.00	4.08	87.67	100.0	100.0	92.89
Mean			50.01	46.06	50.72	43.84	50.01	53.89	49.24
L. peruvianum CMV-INRA	Cotyledon	0.00	0.00	27.77	9.26	100.0	100.0	72.22	90.74
	Hypocotyl	10.00	11.67	0.00	7.23	90.00	88.33	100.0	92.78
Mean			5.84	13.89	8.25	95.00	94.17	86.11	91.76
L. escu. PI174263	Cotyledon	84.44	0.00	0.00	28.15	15.55	100.0	100.0	71.85
	Hypocotyl	0.00	0.00	0.00	0.00	100.0	100.0	100.0	100.0
Mean			0.00	0.00	14.08	57.78	100.0	100.0	85.93
L. escu. var. *ceriaciforme* PI 321749	Cotyledon	37.50	10.00	18.78	22.09	62.57	90.00	81.22	77.93
	Hypocotyl	34.44	13.34	15.00	20.93	65.56	86.67	85.00	79.07
Mean		35.97	11.67	16.89	21.51	64.06	88.33	83.11	78.50
Explant	Cotyledon	58.58	15.98	26.43	33.66	41.44	84.03	73.56	66.34
	Hypocotyl	36.14	16.50	16.58	23.07	63.85	83.51	83.45	76.94
General mean		47.36	16.24	21.51	.....	52.64	83.77	78.51	.....

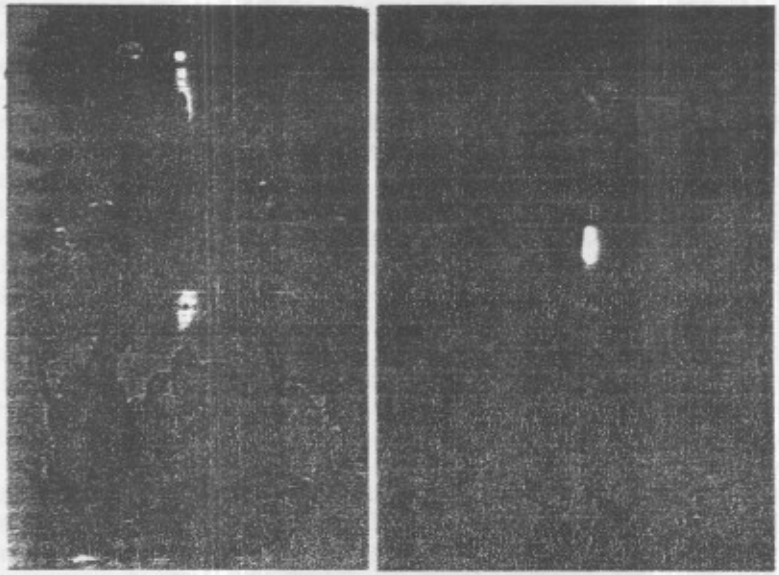
L.S.D at 0.05 for		
Genotype	4.12	4.24
Explant	2.06	2.12
Medium	2.53	2.60
Genotype x Explant.	5.83	5.99
Genotype x Medium	7.14	7.34
Explant x Medium	3.57	3.67
Genotype x Explant x Medium.	10.09	10.38

Table 3. Effect of genotypes, explants and kinetin concentrations on total number of shoots and number of shoots per explant (after five weeks) in tomato.

Genotypes	Explant	Total no. of shoots			Mean	No. of shoots per explant			Mean
		0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN		0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN	
Sarya Hybrid	Cotyledon	0.01	87.67	30.33	39.34	0.01	4.67	4.67	3.11
	Hypocotyl	63.00	80.00	74.67	72.56	3.67	4.00	5.33	4.33
Mean		31.50	83.83	52.50	55.95	1.84	4.33	5.00	3.72
Nematoda Hybrid	Cotyledon	20.67	46.67	113.3	60.22	2.00	2.33	5.67	3.33
	Hypocotyl	22.00	53.33	95.67	57.00	2.00	2.67	5.33	3.33
Mean		21.33	50.00	104.5	58.61	2.00	2.50	5.50	3.33
Mereto Hybrid	Cotyledon	60.00	80.00	100.0	80.00	3.00	4.00	5.00	4.00
	Hypocotyl	8.67	68.67	73.33	50.22	2.00	3.67	4.67	3.44
Mean		34.33	74.33	86.67	65.11	2.50	3.83	4.83	3.72
Abeza Hybrid	Cotyledon	0.01	61.33	106.7	56.00	0.01	3.67	5.33	3.00
	Hypocotyl	0.01	0.01	24.67	8.23	0.01	0.01	2.67	0.90
Mean		0.01	30.67	65.67	32.12	0.01	1.84	4.00	1.95
L. pimpinellifoliu m PI344102	Cotyledon	0.01	0.01	27.00	9.01	0.01	0.01	1.67	0.56
	Hypocotyl	41.33	86.67	120.0	82.67	2.33	4.33	6.00	4.22
Mean		20.67	43.34	73.50	45.84	1.72	2.17	3.83	2.39
L. peruvianum CMV-INRA	Cotyledon	73.33	100.0	96.0	89.78	4.00	5.00	6.67	5.22
	Hypocotyl	31.33	64.67	140.0	78.67	3.00	3.67	7.00	4.56
Mean		52.33	82.33	118.0	84.22	3.50	4.33	6.83	4.89
L. escu. PI174263	Cotyledon	13.00	106.7	160.0	93.22	4.33	5.33	8.00	5.89
	Hypocotyl	73.33	100.0	140.0	104.4	3.67	5.00	7.00	5.22
Mean		43.17	103.3	150.0	98.83	4.00	5.17	7.50	5.56
L. escu. var. *ceriaciforme* PI 321749	Cotyledon	50.67	66.00	96.33	71.00	4.00	4.67	6.00	4.89
	Hypocotyl	56.33	81.00	102.3	79.89	4.33	4.67	6.00	5.00
Mean		53.50	73.50	99.33	75.44	4.17	4.66	6.00	4.94
Explant	Cotyledon	27.21	68.54	91.21	62.32	2.17	3.71	5.38	3.75
	Hypocotyl	37.00	66.79	96.33	66.71	2.63	3.50	3.50	3.87
General mean		32.11	67.67	93.77	.....	2.40	3.51	5.44	.....

L.S.D at 0.05

Genotype	9.66	0.57
Explant	4.83	o.29
Medium	5.92	0.35
Genotype x Explant.	13.67	0.81
Genotype x Medium	16.74	0.99
Explant x Medium	8.36	0.49
Genotype x Explant x Medium.	23.67	1.40



(Feg.1) Regeneration shoots developed from explant at various concentration of ki (A: 0.5 mg/l ki ; B:1.0 mg/l ki.; C: 2.0 mg/l ki)

Plant height and number of leaves per plant.

Data presented in Table 4 indicates that, longer shoots were produced in cvs. Mereto and Nematoda. Maximum number of leaves per plant were produced in cvs. Sarya, Abeza and *L. escu.* PI 174263. significant differences between the explants in plant height, culturing hypocotyl explants produced maximum number of leaves per plant. The medium containing 2.0 mg/l KIN induced longer shoots and maximum number of leaves per plant. A significant effect was observed for the interaction between genotype and explant on plant height in cvs. Sarya, Nematoda, Abeza, *L. Pimpin.* and *L. escu. var ceraciforna*, concerning number of leaves per plant, insignificant differences were observed between cvs. Nematoda, Mereto and *L. escu. Var ceraciforna* .Concerning the interaction between genotypes and media, for all genotypes, the highest plants were produced by using MS medium with 2.0 mg/l KIN except in *L. escu.* PI 174263 and *L. esc. var. ceraciforna.* Concerning the number of leaves per plant, insignificant interaction effect was found between cvs. *L. peruv., L. escu.* PI 174263 and *L. escu. var ceraciforna.* The results of three-way interaction (genotype x explants x medium) revealed that the longest plants were produced by culturing cotyledon explants of cv. Sarya on MS media with 2.0 mg/l KIN and Nematoda on MS with 0.5 KIN.

In general, hypocotyl and cotyledon as a source of explant, *L. escu.* PI 174263 as a variety and MS containing 2.0 mg/l KIN as a culture medium were more effective for the regeneration.

Genotypic variation was observed for all the characteristics studied. Genotypes that exhibited the highest regeneration frequencies did not necessarily produce the highest number of shoots. The low regeneration percentages coupled with limited shoot proliferation reflect the recalcitrant nature of some genotypes to *in vitro* culture. The regeneration was achieved in all genotypes (Table, 2), there were differences among cvs. in the percentage of explants that produced callus and shoots. The highest percentage of callus was produced in cv. Abeza and *L. pimpin.* PI344102. While the highest percentage of shoots was produced in *L. escu.* PI174263.. Results of this study are in line with those reported by Gorbatenko (1990), who found that some genotypes of tomato produced callus and shoots easily, whereas others produced roots readily. Compared to cultivated tomato, its wild counterparts such as *L. pimpin.*, *L. peruv.* and *L. glandulosum* show better regeneration capabilities (Lech *et al.*, 1996). Leaf explants of *L. peruv.* demonstrated higher morphogenic potential than *L. escu.*, while the response of another wild relative of tomato *Solanum pennellii* varied with the type of medium used (Tal *et al.*, 1977). Lech *et al.* (1996) found that *L. peruv.* not only showed better morphogenic potential, but it also responded quickly (2 weeks earlier) compared to *L. esculentum* (Lech *et al.*, 1996). Protoplast cultures of various *Lycopersicon* spp. show similarity in their response to intact explants. Muhlbach (1980) attempted to regenerate protoplasts derived from the leaves of wild *L. peruv.* and cultivated tomato *L. esculentum*,and found that under the same conditions, *L. peruv.* regenerated successfully but not the *L. escu.* in *L. hirsutum*, not all the genotypes show high regeneration capacity. Shoot morphogenic response in *L. hirsutum* extends from the exceptional, with numerous shoots produced by some genotypes, to the recalcitrant, with no shoots being produced by the others (Stommel and Sinden, 1991). The effect of plant genotype on in vitro culture of tomato plants was also reported by Tal *et al.*, (1977) and Padmanabhan *et al.* (1974).

Most genotypes of tomato respond uniquely to plant growth regulators (PGR) during regeneration (Kurtz and Lineberger, 1983). Variations in quantity and type of PGRs influence both the percentage of explants responding, and the number of shoots produced by an explant (Plastira and Perdikaris, 1997). These differences are heritable and may be governed by
both cytoplasmic and nuclear genes, as illustrated in the reciprocal hybrids developed by Ohki *et al.* (1978). Genotypic differences can be seen for the requirements of PGR and the type of explant. Frankenberger *et al.* (1981a, b) showed genotypic influences on regeneration. Davis *et al.* (1994) reported that the genotype 'Better Boy' regenerated only from hypocotyl, whereas 'Spring Giant' regenerated from both hypocotyl and cotyledonary explants.

The high organogenetic competence of *L. peruvianum* and *L. chilense* was reported earlier (Kut and Evans, 1982). The occurrence of *L. hirsutum* accessions ranging from very recalcitrant (Kut and Evans, 1982; Stommel and Sinden, 1991) to highly organogenetic competent (Stommel and Sinden, 1991) have been reported. Competence in *L. peruv.*, Koornneef

et al. (1987) found that this character was associated with two major dominant genes (named Rg-1 and Rg-2). The Rg-1 gene is sufficient for shoot initiation in cultured roots. the best response was observed for *L. chilense* and *L. peruv.* as compared with *L. hirsutum* and *L. escu.* (Lazaro *et al.*, 2001)

The results of three ways interaction (genotype x explants x medium) revealed that the maximum shoots percentages were formed from cotyledon and hypocotyl explants (Table, 2). Earlier studies reported that the use of cotyledon explants of tomato as the most sutible explant source for shoots (Davis, *et al.*, 1994; Ye *et al.*, 1994; Hamza and chupeau, 1993; Plastira and Perdikaris 1997 and Costa *et al.*, 2000a) and callus (Pongtongkam *et al.*,1993). In other studies, hypocotyl was used for direct shoot production (Davis *et al.*, 1994; Plastira and Perdikaris 1997; Zelcer *et al.*, 1984; Gunay and Rao 1980; Chen *et al.*, 1999; Venkatachalam *et al.*, 2000) .The type of explants used not only determines the proportion of explants, which show organogenesis, but also the number of shoots produced per explant. Duzyaman *et al.* (1994) found that the degree of shoot regeneration was in the order of leaves≥cotyledons≥hypocotyls, and all genotypes responded similarly. Plastira and Perdikaris (1997) reported that differential regeneration frequency of various explants in the order of hypocotyl>cotyledon>leaf. Preferential regeneration was also demonstrated findings, Schutze and Wieczorrek (1987) reported *in vitro* shoot production from cotyledon explants was better than that from hypocotyl explants. Most tissues of tomato seem to have high totipotency; however the choice of the right explant may vary with the genotype. The specific 61-kd protein was found only in cotyledons, this protein might play an important role in the morphogenesis of tomato organs (Shan *et al.*, 2004)

In the present investigation, maximum callus and shoot induction was observed on MS salts and B5 vitamin. Maximum callus was observed on MS media with 0.5 mg /l KIN , as well as the maximum shoot induction was produced on MS media with 1.0 mg/l or 2.0 mg /l KIN (Tables 2, 3 and 4). B5 vitamins along with MS basal media were successfully used by Selvi and Khadar (1993). Four major cytokinins (Zeatin, 2ip, BA and KIN can be used either separately or in combination with auxins for organogenesis in tomato (Poonam *et al.*, 2005). Santana and Ramirez (1989); Pongtongkam *et al.* (1993) Chandel and Katiyarz (2000); Ramiah and Rajappan (1996) and Chandra *et al* . (1995) used KIN (0.1 – 2.0 mg/l) to induce adventitious shoot from tomato explant.

In the present study, shoots formed roots on MS media free hormone. Nguyen *et al.* (1992) studied the steroid glycosides for their PGR-like properties on tomato tissue culture, and found that optimum PGR for tomato is genotypic dependent, however plus treatment of PGR in general is not found to be beneficial for rooting. Tomato contains high levels of endogenous phytohormones and thus it does not require higher concentrations of auxins for rooting (Mensuali-Sodi *et al.*, 1995).

Table 4. Effect of genotypes, explants and kinetin concentrations on plant height and number of leaves per plant (after five weeks) in tomato.

Genotypes	Explants	Plant height(cm)			Mean	No. of leaves per plant			Mean
		0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN		0.5 mg/l KIN	1.0 mg/l KIN	2.0 mg/l KIN	
Sarya Hybrid	Cotyledon	0.01	3.00	5.66	2.89	0.01	2.33	3.00	1.78
	Hypocotyl	3.00	3.33	3.67	3.33	2.33	2.33	2.33	2.33
Mean		1.51	3.17	4.67	3.11	1.17	2.33	2.66	2.06
Nematoda Hybrid	Cotyledon	4.67	2.33	4.67	3.89	1.67	1.67	2.33	1.89
	Hypocotyl	2.00	3.00	4.33	3.11	1.67	1.67	2.33	1.89
Mean		3.33	2.67	4.50	3.50	1.67	1.67	2.33	1.89
Mereto Hybrid	Cotyledon	4.00	3.00	4.33	3.78	1.67	1.67	2.00	1.78
	Hypocotyl	3.33	3.33	3.67	3.44	1.67	1.00	2.00	1.56
Mean		3.67	3.17	4.00	3.61	1.67	1.33	2.00	1.67
Abeza Hybrid	Cotyledon	0.01	4.33	5.33	3.23	0.01	1.67	2.33	1.34
	Hypocotyl	0.01	0.01	3.67	1.23	0.01	0.01	2.67	0.90
Mean		0.01	2.17	4.50	2.23	0.01	0.84	2.50	1.12
L. pimpinellifolium PI344102	Cotyledon	0.01	0.01	3.67	1.23	0.01	0.01	1.33	0.45
	Hypocotyl	2.33	2.67	3.33	2.78	1.67	2.33	1.33	1.78
Mean		1.72	1.38	3.50	2.00	0.84	1.17	1.33	1.11
L. peruvianum CMV-INRA	Cotyledon	3.33	3.33	4.00	3.56	1.67	0.01	1.33	1.00
	Hypocotyl	3.33	3.00	3.00	3.11	2.33	1.33	1.67	1.78
Mean		3.33	3.17	3.50	3.33	2.00	0.67	1.50	1.39
L. escu. PI174263	Cotyledon	3.00	2.00	2.33	2.44	2.00	1.67	2.00	1.89
	Hypocotyl	2.67	2.33	2.00	2.33	2.00	2.00	2.00	2.00
Mean		2.83	2.17	2.17	2.39	2.00	1.83	2.00	1.94
L. escu. var. *ceriaciforme* PI321749	Cotyledon	3.33	1.33	1.67	2.11	2.00	1.67	1.67	1.78
	Hypocotyl	2.00	1.67	1.33	1.67	1.00	1.67	2.00	1.56
Mean		2.67	1.50	1.50	1.89	1.50	1.67	1.83	1.67
Explant	Cotyledon	2.30	2.42	3.96	2.89	1.30	1,34	2.00	1.49
	Hypocotyl	2.34	2.42	3.13	2.63	1.59	1.54	2.04	1.72
General mean		2.32	2.42	3.54	.....	1.36	1.44	2.02	.....

L.S.D at 0.05		
Genotype	0.48	0.39
Explant	0.24	0.19
Medium	0.29	0.24
Genotype x Explant.	0.68	0.55
Genotype x Medium	0.83	0.68
Explant x Medium	0.42	0.34
Genotype x Explant x Medium.	1.18	0.96

Experiment 2:

In the present study four tomato wild genotypes (Table, 1) were subjected to gradual increase in sea salt concentrations (0.0, 2000, 4000, 6000 and 8000 ppm) for 30 days in order to test salinity tolerance in tomato.

In general increasing levels of salinity in the germination media progressively decreased germination percentage, plant height, root length, leaves number and plant fresh weight (Table, 5).

The main differences among cvs. were found in these parameters. The germination percentage and plant fresh weight was the highest in *L.escu.*

PI174263. Tallest plants were produced in *L.escu.* PI 174263 and *L.escu. var ceriaciforme* PI3217. Longest roots were found in *L. Pimpin.* and *L. peruv.* There were insignificant differences among cvs. in leaves number.

Concerning the interaction effect between the salinity level and genotypes, increasing the salinity reduced germination percentage, plant height, leaves number and plant fresh weight in all genotypes, except in *L. Pimpin.* increase the salinity up to 4000 ppm increased plant height. Furthermore, data of root length indicated that, the initial in salinity levels decreased the root length, while the successive increasing in salinity increased root length in *L. Pimpin.* at 4000 and 8000 ppm, in cv. *L. escu.* PI 174263 at 2000 ppm and in *L. escu.* var. *ceriaciforme PI*3217 at 6000 ppm.

Table 5. Effect of salinity levels on germination percentage, plant height, root length, leaves number and plant fresh weight (after five weeks) in wild tomato.

Genotypes	Salinity level (ppm.)	Germination %	Plant height (cm)	Root length (cm)	Leaves number	Plant weight (gm)
L. pimpinellifolium PI344102	Zero	100.00	14.00	7.33	4.67	0.63
	2000	100.00	12.67	7.00	4.33	0.65
	4000	80.00	15.33	9.00	4.33	0.45
	6000	73.33	6.00	7.00	3.33	0.15
	8000	56.67	4.67	8.67	2.67	0.04
Mean		82.00	10.53	7.80	3.87	0.38
L. peruvianum CMV-INRA	Zero	100.00	12.33	8.00	6.00	0.48
	2000	100.00	11.67	7.00	5.67	0.42
	4000	93.33	11.33	7.67	3.33	0.25
	6000	63.33	7.00	7.00	3.00	0.15
	8000	50.00	7.00	6.33	2.00	0.13
Mean		81.33	9.87	7.20	4.00	0.28
L. escu. PI174263	Zero	100.00	15.67	7.00	4.67	2.10
	2000	100.00	15.00	8.67	4.67	2.38
	4000	100.00	13.67	7.67	3.67	0.84
	6000	90.00	9.67	4.33	3.67	0.47
	8000	80.00	8.00	5.00	3.00	0.44
Mean		94.00	12.40	6.93	3.93	1.25
L. escu. var. ceriaciforme PI321749	Zero	100.00	19.67	5.00	6.33	0.79
	2000	100.00	19.33	5.00	6.00	1.05
	4000	76.67	12.33	5.00	4.00	0.33
	6000	70.00	8.33	6.00	3.67	0.41
	8000	50.00	4.33	2.67	2.00	0.12
Mean		79.33	12.80	4.73	4.40	0.54
Salinity means	Zero	100.00	15.42	6.83	5.42	1.00
	2000	100.00	14.67	6.50	5.17	1.13
	4000	87.50	13.17	7.58	3.83	0.46
	6000	74.17	7.75	6.92	3.42	0.29
	8000	59.17	6.00	5.50	2.42	0.18
L.S.D at 0.05 for	cvs.	3.64	1.24	.0.92	0.83	0.41
	salinity	4.07	1.39	1.03	0.93	0.46
	cvs. X salinity	8.13	2.28	2.07	1.85	0.93

Increasing the salinity reduced germination percentage, plant height, leaves number and plant fresh weight in all genotypes, except in *L. Pimpin.* where increasing the salinity to 4000ppm increased plant height. Furthermore, data of root length indicated that, the initial in salinity levels decreased the root length, while the successive increasing in salinity increased root length in *L. Pimpin.* at 4000 and 8000 ppm , in cv. *L. escu.* PI 174263 at 2000 ppm and in *L. escu. var cerlaciforme PI*3217 at 6000 ppm (Table, 5). For several plant species grown *in vivo*, including tomato, leaf growth has been more sensitive to salinity than root growth (Salim, 1989 Perez- Alfocea *et al.,* 1994). Root growth has been found to be more adversely affected than leaf growth by an increasing supply of NaCl (Mills, 1989; Bourgeais and Guerrier, 1992; Sweby *et al.*, 1994). Similar results were obtained in this work: although both root and leaf growth were inhibited by slat, the effects were more pronounced on root growth mainly in *L. escu.*. Higher salt tolerance has been reported in wild tomato species, including the accessions used in this work, than in cultivated tomato. In this work, higher salt tolerance was noticed in *L. pimin.* as compared to *L. escu.* this was clearly shown for plant height, leaves number and root length at the salinity level of 4000 ppm sea slates(Table, 6). Thus, on the basis of reduction of plant FW with increasing salinity, the salt tolerance of *L .escu.* was higher than that of *L .pimpin.* and *L. peruv.* It may be concluded that root growth and plant height are good characteristics for evaluating salt tolerance of tomato species through *in vitro* culture.

REFERENCES

Bhatia, P. (2004).Regeneration, micropropagation and somatic embryogenesis in tomato (*Lycopersicon esculentum* Mill.). Ph.D. thesis, Central Queensland University, Rockhampton, Australia.

Bourgeais-Chaillou, p. and G. Guerrier (1992). Salt response in *Lycopersicon esculentum* calli and whole plants. J . Plnt Physiol. 140: 494-501.

Cano, E.A.; F. Pérez-Alfocea; V. Moreno; M. Caro and M.C. Bolar (1998). Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant Cell Tiss. Org. Cult. 53: 19–26.

Cano, EA. ; F. Pérez-Alfocea; V. Moreno, and M.C. Bolar (1996). Responses to NaCl stress of cultivated and wild tomato species and their hybrids in callus cultures. Plant Cell Rep. 15: 791–794.

Chandel, G. and S. K. Katiyar (2000). Organogenesis and somatic embryogenesis in tomato (*Lycopersicon esculantum* Mill.). Adv. Plant Sci. 13: 11–17

Chandra, R. ; S. Khetrapal; P. Patil; N. Gupta and R. Polisetty (1995). *In vitro* regeneration of hybrid and non-hybrid tomato (*Lycopersicon esculentum* L.). Indian J. Plant Physiol. 38: 139–142.

Chen, H.; J. Zhang ; T. Zhuang and G. Zhou (1999). Studies on optimum hormone levels for tomato plant regeneration from hypocotyl explants cultured *in vitro*. Acta Agric. Shanghai 15: 26–29.

Costa, M. G. .; F. T. S. Nogueira; M. L.Figueira; W. C. Otoni; S. H. Brommonschenkel and P. R. Cecon (2000a). Influence of the antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum* Mill.) genotypes.Plant Cell Rep. 19:327 – 332.

Costa, M. G.; F. T. S. Nogueira and W. C. Otoni (2000b). Brommonschenkel, S. H. *In vitro* regeneration of processing tomato (Lycopersicon esculentum Mill.) 'IPA-5' and 'IPA-6'. Ciencia Agrotecnol. 24:671 – 678.

Cruz, V; J. Cuartero; M.C. Bolar and M. Romero (1990). Evaluation of characters for ascertaining salt stress responses in *Lycopersicon* species. J. Amer. Soc. Hort. Sci. 115: 1000–1003.

Davis, D.G; K.A. Breiland; D.S. Frear and G.A. Secor (1994). Callus initiation and regeneration of tomato (*Lycopersicon esculentum*) genotypes with different sensitivities to metribuzin. Plant Growth Regul. Soc. Am. Quart. 22: 65–73.

Duzyaman, E; A. Tanrisever and G. Gunver (1994). Comparative studies on regeneration of different tissues of tomato *in vitro*. Acta Horti: 235–242.

El-Farash, E. M.; H. I. Abdalla; A. S. Taghian and M. H. Ahmad (1993). Genotype, explant age and explant type as affecting callus and shoot regeneration in tomato Assiut J. Agric. Sci. 24:3 – 14.

Epstein, E. (1976). Genetic potentials for solving problems of soil mineral stress: Adaptation of crops to salinity. In: Wright MJ (ed.) Plant Adaptation to Mineral Stress in Problem Soils (pp 73–82). Cornell University, New York.

Faria R.T. and R.D. Illg (1996). Inheritance of *in vitro* plant regeneration ability in the tomato. Rev. Brasil. Genética 19: 113–116.

Frankenberger, E.A; P.M.Hasegawa and E.C. Tigchelaar (1981a). Influence of environment and developmental state on the shoot forming capacity of tomato genotypes. Z. Pflanzenphysiol. 102: 221–232.

Frankenberger, EA; PM. Hasegawa and E.C. Tigchelaar (1981b). Diallel analysis of shoot-forming capacity among selected tomato genotypes. Z. Pflanzenphysiol. 102: 233–242.

Gamborg, O; R. K. Miller and K. Ojima (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell. Res. 50: 151–168.

Garcia- Reina, G; Moreno, V. and A. Luque (1988). Selection for NaCl tolerance in cell culture of three Canary Island tomato land races. I. Recovery of tolerant plantlets from NaCl-tolerant cell strains. J. Plant Physiol. 133: 1–6.

Gorbatenko, I. Y. (1990). Micropropagation of different tomato genotypes *in vitro*. Doklady Vsesoyuznoi Ordena Lenina i Ordena Trudovogo Krasnogo Znameni Akademii Sel'skokhozyaistvennykh Nauk Imeni 18 – 22.

Gunay A.L. and P.S. Rao (1980). *In vitro* propagation of hybrid tomato plants (*Lycopersicon esculentum* L.) using hypocotyl and cotyledon explants. Ann. Bot. 45: 205–207.

Hamza, S. and Y. Chupeau (1993). Re-evaluation of conditions for plant regeneration and *Agrobacterium*-mediated transformation from tomato (*Lycopersicon esculentum*). J. Exp. Bot. 44: 1837–1845.

Koornneef, M; C.J. Hanhart and L. Martinelli (1987). A genetic analysis of cell culture traits in tomato. Theor. Appl. Genet. 74: 633–641.

Koornneef, M; J. Bade; C. Hanhart; K. Horsman; J. Schel; W. Soppe; R. Vekerk and P. Zabel (1993). Characterization and mapping of a gene controlling shoot regeneration in tomato. Plant J. 3: 131–141.

Kurtz, S.M. and R.D. Lineberger (1983). Genotypic differences in morphogenic capacity of cultured leaf explants of tomato. J. Am. Soc. Hort. Sci. 108: 710–714.

Kut, S. A. and D. A. Evans (1982). Plant regeneration from cultured leaf explants of eight wild tomato species and two related *Solanum* species. *In vitro* 18: 593–598.

Lazaro, E. P. P; G. M. Patricia; V. Claudia; E. K. Jane and V.S. Marie-Anne (2001). Shoot regeneration capacity from roots and transgenic hairy roots of tomato cultivars and wild related species. Plant Cell, Tissue and Organ Culture 65: 37–44.

Lech, M; K. Miczynski and A. Pindel (1996). Comparison of regeneration potentials in tissue cultures of primitive and cultivated tomato species (*Lycopersicon* sp.). Acta Soc. Bot. Poloniae 65: 53–56.

Martinez, C.A; M. Maestri and E.G. Lani (1996). *In vitro* salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. Plant Sci. 116: 177–184.

McCoy, T.J. (1987). Tissue culture evaluation of NaCl tolerance in *Medicago* species: cellular versus whole plant response. Plant Cell Rep. 6: 31–3.

Mensuali-Sodi, A; M. Panizza and F. Tognoni (1995). Endogenous ethylene requirement for adventitious root induction and growth in tomato cotyledons and lavandin microcuttings *in vitro*. Plant Growth Regul. 17: 205–212.

Meredith, C.P. (1979). Shoot development in established callus cultures of cultivated tomato (*Lycopersicon esculentum* Mill.). Z. Pflanzenphysiol. Bd. 95: 405–411.

Mills, D. (1989). Differential response of various tissue of *Asparagus officinalis* to sodium chloride. J. Exp. Bot. 40: 485-491.

Morgan, A. and E.C. Cocking (1982). Plant regeneration from protoplasts of *Lycopersicon esculentum* Mill. Z. Pflanzenphysiol. Bd. 106: 97–104 reaction. Methods Enzymol. 155: 335–350.

Muhlbach, H. P. (1980). Different regeneration potentials of mesophyll protoplasts from cultivated and a wild species of tomato. Planta 148: 89–96

Murashige,T. and F. Skoog (1960). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 5:473-497.

Nguyen, H.M; N.N Balashova; P.K kintya; V. A. Smirnov and T.T. kieu (1992). Effects of steroid glycosides on tomato tissue culture. Sel'Skokhozyaistvennaya Biologiya 57–63.

Ohki, S; C. Bigot and J. Mousseau (1978). Analysis of shootforming capacity *in vitro* in two lines of tomato (*Lycopersicon esculentum* Mill.) and their hybrids. Plant Cell Physiol. 19: 27–42.

Padmanabhan, V.; E. F. Paddock and W. R. Sharp (1974). Plantlet formation *in vitro* from *Lycopersicon esculentum* leaf callus Can. J. Bot. 52:1429 – 1432.

Pérez-Alfocea, F; G. Gerrier; M.T. Esta', and M. C. Bolar (1994). Comparative salt responses at cell and whole-plant levels of cultivated and wild tomato species and their hybrid. J. Hort. Sci. 69: 639–644.

Plastira, V.A. and A.K. Perdikaris (1997). Effect of genotype and explant type in regeneration frequency of tomato *in vitro*. Acta Horti. 231–234.

Pongtongkam, P; P. Ratisoontorn; S. Suputtitada ; S. Piyachoknagul; L. Ngernsiri and A.Thonpan (1993). Tomato propagation by tissue culture. Kasetsart J. 27: 269–277.

Poonam, B.; A. Nanjappa and G. M. David (2005). Effect of genotype, explant orientation, and wounding on shoot regeneration in tomato. *In vitro* Cell. Dev. Biol. Plant 41:457-467.

Ramiah, M. and K. Rajappan (1996). Direct shoot regeneration from excised cotyledonary leaf of tomato. South Indian Hort. 44: 101–102

Salim, M. (1989). Effect of salinity and relative humidity on growth and ionic relations of plants. New Phytol. 113: 13-20.

Santana, N. and A.L. Ramirez (1989). Influence of NAA, IAA and kinetin on morphogenesis of leaf tissue of tomato (*Lycopersicon esculentum*, Mill.) cultured *in vitro*. Cultivos Tropicales 11: 63–67.

Saranga, Y; D. Zamir ; A. Marani and J. Rudich (1993). Breeding tomatoes for salt tolerance: variations in ion concentrations associated with response to salinity. J. Amer. Soc. Hort. Sci. 118: 405–408.

Schutze, R. and G. Wieczorrek (1987). Investigations into tomato tissue cultures. I. Shoot regeneration in primary explants of tomato. Arch. Zuchtungsforschung 17: 3–15.

Selvi, D.T. and M.A. Khader (1993). *In vitro* morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.) var. PKM.1. South Indian Hort. 41: 251–258.

Shan, H.Y.; X. W. Li; D. Li; S. Q. Shao and P. Liu (2004). Differential expression of specific proteins during *in vitro* tomato organogenesis. Russian J. of Plant Physio., 51, 3 :379-385.

Steel, R.G. and J. H. Torrie (1960). Principals and procedures and statistics A biometrical approach. McGraw-Hill Book Co.NewYork.

Stommel, J. R. and S. L. Sinden (1991). Genotypic differences in shoot-forming capacity of cultured leaf explants of *Lycopersicon hirsutum*. HortScience 26:1317 – 1320.

Sweby, D. L; B. I. Huckett and M.P. Watt (1994). Effect of nitrogen nutrition on salt stressed *Nicotiana tabacum* var. Samsun *in vitro* plantlets. J. Exp. Bot. 45: 995-1008.

Tal, M. (1984). Physiological genetics of salt resistance in higher plants: studies on the level of the whole plant and isolated organs, tissues and cells. In: Staples RC and Toenniessen GH (eds) Salinity Tolerance in Plants. Strategies for Crop Improvement (pp 301–320). Wiley and Sons, New York

Tal, M.; H. Heikin and K. Dehan (1978). Salt tolerance in the wild relatives of the cultivated tomato: responses of callus tissue of *Lycopersicon esculentum*, *L. peruvianum* and *Solanum pennellii* to high salinity. Z. Pflanzenphysiol. 86: 231–240.

Tal, M.; K. Dehan and H. Heikin (1977). Morphogenetic potential of cultured leaf sections of cultivated and wild species of tomato. Ann. Bot. 41:937 – 941.

Venkatachalam, P; N. Geetha; P. Priya ; G. Rajaseger and N. Jayabalan (2000). High frequency plantlet regeneration from hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.) via organogenesis. Plant Cell Biotechnol. Mol. Biol. 1: 95–100.

Ye, Z. B; H. X. Li and G. L. Zhou (1994). *In vitro* culture of tomato cotyledons and regenerated plants. J. Huazhong Agric. Uni. 13: 291–295.

Zelcer, A; O. Soferman and S. Izhar (1984) An *in vitro* screening for tomato genotypes exhibiting efficient shoot regeneration. J. Plant Physiol. 115: 211–215.

تاثير التركيب الوراثي والجزء النباتي وتركيزات الكينتين علي اعادة توليد الافرع وقدرتها على تحمل الملوحة في الطماطم

سحر سميح طه

قسم الخضر كلية الزراعة ، جامعة القاهرة

أجريت هذه الدراسة علي٤ هجن من الطماطم (ساريا – نيما تودا – ميرتيو – ابيـــزا) و٤ انوع برية فصلت السويقة الجنينية السفلي والاوراق الفلقية ثم زرعت علي بيئة مور اشيجى و سكوج تحتوي علي ٣% سكروز + فيتامينات بيئة جمبورج +١% اجار ومضاف اليها ٠,٥ ، ١ ، ٢ ملجم / لتر كينيتن . اعلي نسبة انتاج الكالس في الهجين ابيزا و *L. pimpin* PI 1344102 واعلي عدد من الاجزاء النباتية انتاجا للافـرع الخـضرية فـي النـوع البـري *L.escu.* PI 174263 علي بيئة موراشيجى وسكوح مضاف اليها ١ ، ٢ ملجـم كينتـين/ لتـركنيتين. تـم الحصول علي اعلي عدد من الافرع الخضرية للجـزء النبـاتي بزراعـة الاوراق الفلقيـة مـن *L.escu.* PI 174263 علي بيئة موراشيجى وسكوح مضاف اليها ٢ ملجـم / لتـر كينتـين. زرعت بذور الطماطم من الانواع البرية الاربعة علي بيئة موراشيجى وسكوح مـضاف اليهـا ٢ ملجم / كينتين مضافا اليها تركيزات من املاح البحر (صـفر ، ٢٠٠٠ ، ٤٠٠٠، ٦٠٠٠، ٨٠٠٠ جزء في المليون). اعلي نسبة انبات ووزن طازج لوحظ فـي النـوع البـري *L.escu.* PI 174263. اطول النباتات من النوع البري *L.escu.* PI 174263 والنوع البـري *L.escu* var.*cariacifome*. وزيادة الملوحة ادت الي نقص نسبة الأنبات وارتفاع النبات وعدد الاوراق والوزن الطازج لنبات في كل الانواع المستخدمة فيما عدا النوع *L. pimpin* PI 1344102 زيادة الاملاح حتي ٤٠٠٠ جزء من مليون ادت الي زيادة ارتفاع النبات.