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Effect of the Chemical Mutagens Sodium Azide on Plant Regeneration of Two Tomato Cultivars under Salinity Stress Condition *in vitro*

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Abstract: The study was carried out to induce variations and stimulate callus induction, plant regeneration from different explants of two tomato (*Lycopersicon esculentum* Mill.) cultivars Trescantos and super Regina by using tissue culture technique and Sodium azide as a chemical mutagens at concentrations (0.0, 2.0 and 4.0) mM under salinity stress condition at the levels(3.0, 6.0 and 9.0) dS/m. Different plant growth regulators were tested for their potentials in callus induction. The results revealed that treated seeds with SA (sodium azide) at concentration (2.0) mM increased seed germination percentage, seedling height and root length as compare to control treatment. While (4.0) mM concentration cause a reduction in all parameters mentioned above. Concerning to callus induction both cultivars showed a different response against different tested media with varying concentrations of plant growth regulators and despite their variable response to all tested media a combination of (2.0) mg from Kinetin (KIN) and Indol acetic acid (IAA) was found to be the most effective as compare to other treatments. Moreover, when callus transferred to a stressed media the variation was observed in explants fresh weight, and high reduction with the increment of salt level were recorded. Similarly the regeneration efficiency from stressed callus were observed at the level 3.0 and 6.0 dS/m while 9.0 dS/m the callus failed to regenerate plants for all three explants of both tomato cultivars.

Key words: Tomato, callus, salinity, *in vitro.*

1. Inroduction

The cultivated tomato (*Lycopersicon esculentum* Mill.) which belongs to the family Solanaceae considered as the 2nd most important vegetable crop in the world after potato[1-3] and according to [4] tomato ranks third among vegetable crops with an annual production of 283 million metric tones in the year 2009.

Tomato have a nutritional and medicinal value for its content from vitamin A and C, [5] besides carotenoids pigments which conceders as an antioxidant protect humans body from free radicals damages and reduce the risk of getting cancer and also cholesterol free [6, 7] and based on [8] tomato sometime rightly referred to as poor man's orange .

During the past few years Iraq lands had witnessed an adverse environmental stress such as saline and drought conditions which affected negatively on crop yields [9]. Due to its importance as vegetable and medicinal value, many laboratories are doing research on tomato and other different economic crops to improve their production under abiotic stresses by using chemical or physical mutagens in corporate with *in vitro* technique [10-13]. Since several researches proved that Sodium azide (NaN3) was very effective in inducing mutation with respect to tomato[14, 15] so the aim of this study is improvements of two tomato

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cultivars for plant regeneration under salinity stresses condition by using tissue culture technique in cooperated with mutation technique.

2. Materials and Methods

The study was conducted during the period 2010-2011 in the tissue cultures lab. Ministry of science and Technology.

Surface sterilization and establishment of aseptic seedling from treated seeds. Seeds of two tomato cultivars (Trescantos and super Regina) were soaked for four h with sodium azide (NaN3) as a chemical mutagen to increase the genetic variation at concentration (0.0, 2.0 and 4.0) mM. The treated seeds were washed three times to remove excess NaN3 with autoclaved distilled water. Subsequently, treated and untreated seeds were surface disinfected with ethanol at concentration 70% for 2 min, followed sodium hypochlorite at concentrations (6%) for 20 min and rinsed for 15 min with sterilized distilled water three times (5 min each time). The treated and untreated seeds for both cultivars were germinated on full strength of inorganic salts [16] medium solidified with 7.0 g/1Agar, the culture were incubated at 25 ± 2 °C under cool-white fluorescent light for three weeks.

3. Callus Induction

Callus initiation from hypocotyls, epicotyls and cotyledon leaves explants was achieved on Murashige, and Skoog medium [16] supplemented with thiamine HCl, pyridoxine HCl, nicotinic acid, glycine, inositol and sucrose at concentrations: (0.1, 0.5, 0.5, 2.0, 100 and 30000)mg/L respectively. To achieve callus induction from different explants of both tomato cultivars, different plant hormones were added to the media that is (T1, T2, T3, T4) which represent (0.5 mg/L kinetin + 0.5 mg/L IAA) (1.0 mg/L kinetin + 1.0 mg/L IAA) (1.5 mg/L kinetin + 1.5mg/L IAA) (2.0mg/L kinetin + 2.0mg/L IAA). The pH of medium was adjusted to 5.75 then media were solidified with 6 gm/L agar before autoclaving at 120 °C for 20 min. The culture were incubated at 25 ± 2 °C under cool-white fluorescent light, after 4 weeks callus fresh weight were recorded. Application of NaCl treatments and plant regeneration.

To Study the effect of salt stress on callus growth and plant regeneration from stressed callus after four weeks from callus proliferation a 100 mG fresh weight from the callus of each two tomato cultivars explants were cultured on 1/2 MS (half strength) media supplemented with the optimum treatment from previous media for callus induction and three levels of salt (NaCl) that is (3.0, 6.0 and 9.0) dS/m. Cultures were incubated at 25 ± 2 °C under cool-white fluorescent light conditions for 6 weeks. The stressed callus were transferred to a regeneration media containing MS nutrient salts supplemented with 100 mg/1 myo-inositol, 3 mg/L^{-} Banzyl amino purine (BAP), 30 g/L sucrose and 6 g/1 Agar. The statistical analysis was performed using C.R.D (completely randomized design) with six replicates for each parameter was used, and the means were compared using (GenStat 12ed) program with L.S.D (least significant differences) at 0.05 level.

4. Results

Following 3 weeks of treatment and culturing on MS medium free hormone, the effect of different concentration of NaN3 on %seeds germination, seedling height (cm) and root length (cm) of two tomato cultivars is shown in Table 1. Sodium azide concentrations were affected significantly on both tomato cultivars according to germination percentage, seedling height and root length parameters and the highest seeds germination percentage was observed in 2.0 mM of NaN3 treatment was 81.7%, 86.7% for both tomato cultivars Super regina and Trescantos respectively while less germination percentage was observed 46.7 %, 51.7% when seeds treated with 4.0 mM of NaN_3.

Similarly the results revealed that seedling height and root length of the two tomato cultivars at various

concentration of sodium azide were varied and (2.0 mM) concentration gave the best performance in all the parameters studied. While a higher reduction was found at (4.0 mM) concentration. Sodium azide affects the shoot and root length and also delay in seed germination percentage, similar results were observed on tomato when using high concentration of different chemical mutagenics [17].

The response of different explants for callus induction in the presence of different plants hormones.

The results in Table 2 revealed that adding different concentration of plant growth hormones affected significantly on callus induction and among all different concentrations and combination T4 treatment which represent (2.0 mg/L kinetin + 2mg/L IAA) was found to be the most effective than other treatments for callus induction and with this treatment the highest weight of callus was 251.9 mG, 245.0 mG for super Regina and Trescantos respectively. Furthermore, the results obtained in Table 2 indicate that cotyledon leaves for both cultivars gave negative response as compare to other explants in T1 treatment which represent (0.5 mg/L kintient + 0.5 mg/L IAA). Although the results indicate that the response of both cultivars and there explants were varied and epicotyls produced better callus fresh weights than cotyledonary or hypocotyls explants in both tomato cultivars.

The effect of salt stress on callus fresh weight (mG) and plant regeneration from stressed callus.

Following 6 weeks of exposing a100 mG callus fresh weight for salinity stress conditions the data in Table 3 revealed a significant reduction in callus fresh weight with progressive increasing of salt level and higher reduction was found at the level 9.0 dS/m (135.1 and 166.1) mG for both super Regina and Trescantos respectively.

Moreover, the response of the three explants were varied and concerning to super Regina the higher response reached 250.5 mG for the callus produced from hypocotyls as compare with 238.4, 233.4 mG for epicotyls and cotyledons leaves respectively. While in Trescantos the hypocotyls explants presented the weakest and gave less fresh weight reached 203.0 mG as compare to cotyledons leaves (288.2 mG) and epicotyls (212.1 mG) respectively.

The effect of salt stress on plant regeneration from stressed callus.

Data on the shoot formation from stressed callus of different explants for two tomato cultivars which summarized in Table 4 revealed that Plant regeneration

Table 1 Effects of Sodium azide on %seeds germination, seedling height (cM) and root length(cM) of two tomato cultivars.

NaN3 mM concentration	Germination %		seedling height (cM)		root length (cM)	
	super Regina	Trescantos	super Regina	Trescantos	super Regina	Trescantos
Control	30	48.3	1.41	1.13	11.17	11.67
2.0 mM	81.7	86.7	3.45	2.82	14.67	16.67
4.0 mM	46.7	51.7	1.79	1.64	5.83	6.67
$S.D_{0.05}$	9.42		0.59		1.68	

Table 2 Effect of different treatments on callus fresh weight (mG) from different explants of two tomato cultivars.

treatments	super Regina			Mean	Trescantos			Mean
	cotyledons	hypocotyls	epicotyls		cotyledons	hypocotyls	epicotyls	
T1	0.0	88.4	139.9	76.1	0.0	67.7	85.9	51.2
T2	174.2	162.9	170.7	169.3	52.6	96.8	139.7	96.4
T3	101.3	140.6	185.4	142.4	77.2	129.4	173.4	126.7
T4	168.4	222.0	251.9	214.1	138.2	187.6	245.0	190.3

L.S $.D_{(0.05)}$ explants = 42.91
treatments = 49.55
Interaction= 85.83

L. S. $D_{(0.05)}$ explants = 17.75
treatments = 19.97
Interaction= 29.40

*Each number represents mean fresh weight of six replicates.

Table 3 Effect of salt stress on callus fresh weight (mG) produced from different tomato explants *in vitro*.

Salt levels dS/m	super Regina			Mean	Trescantos			Mean
	cotyledons	hypocotyls	epicotyls		cotyledons	hypocotyls	epicotyls	
3.0	267.2	334	323	308.1	313.7	198.2	225.9	246
6.0	281.7	284.5	271.3	279.2	364.7	238	271.3	291.3
9.0	151.3	133.1	120.9	135.1	186.2	172.9	139	166.1
Mean	233.4	250.5	238.4		288.2	203	212.1	
L.S .$D_{(0.05)}$ explants = 25.49 Interaction = 44.15					explants = 6.97 Interaction = 12.08			
Salt levels = 25.49					Salt levels = 6.97			

*Each number represents mean of six replicates a100 mg of callus.

Table 4 Effects different levels of sodium chloride on shoot formation from stressed callus of different explants for two tomato cultivars.

Salt levels dS/m	super Rejena			Trescantos		
	cotyledons	hypocotyls	epicotyls	cotyledons	hypocotyls	epicotyls
3.0	10.2	12.5	9.2	8.7	7.1	10.1
6.0	18.0	16.3	19.2	14.5	13.4	11.2
9.0	0.0	0.0	0.0	0.0	0.0	0.0
$L.S.D_{(0.05)}$		1.32			2.08	

efficiency was increased with the increase in NaCl levels start from 3.0 up to 6.0 dS/m and maximum plant regeneration percentage was observed at the level (6.0 dS/m) then no plant regeneration was observed at the level (9.0 dS/m).

5. Discussion

Sodium azide has been used in wide range of applications to improve crops ability in their resistance against harmful pathogen [18] or to produce desire variation for salt tolerance and other abiotic stresses, in our research using Sodum azid at various concentration results either negative or positive responses at high or low concentration respectively, in case of seeds germination rate the higher concentration affected on some biological activities such as a specific enzymes which involves in seed germination processes and reduced germination percentages and other growth parameters similar results are agreements with [15, 10, 11].

Concerning to the callus induction in the presence of different combination of plant hormones in the tested media, it is obvious from Table 2, various response was noticed among the different explants and cultivars. However, maximum fresh weight were observed in T4 media which supplemented with (2.0mg/L kinetin + 2.0mg/L IAA). The results are agreement with [19] who stated that enhancing callus induction media with equimolar concentration of auxin and cytokinins produced a good and faster production of callus and disagreement with [20] who revealed no considerable response on callus induction by the IAA and kinetin combination.

For callus fresh weight and plant regeneration under stress conditions all tomato explants for both cultivars were able to produce callus and plant regeneration under various level of NaCl. However, these capacities were varied and a high reduction in callus fresh weight were noticed with progressive increasing in NaCl levels, similar results in reduction of callus fresh weight found at the high level of salinity stress, these results are agreement with [21] and disagreement with [22] who proved a positive response for callus growth in high saline media. Regarding to plant regeneration both cultivars with there different explants were failed to regenerated at high level of NaCl.

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