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SALINITY TOLERANCE STUDIES ON *SOLIDAGO ALTISSIMA* GRAY. BY *IN VITRO* CULTURE

BY

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ABSTRACT

This study had been carried out at the Tissue Culture and Germplasm Conservation Res. Lab., Hort. Res. Institute, (ARC) Egypt, on *Solidago altissima* Var. "Tara" during the period from 2005 to 2006, in order to investigate the influence of different levels of salinity treatments on the *in vitro* propagation and chemical constituents of the explants.

The data revealed that for shootlet proliferation and rooting behaviour stages; 11000 ppm salinity lead to kill all the explants in the 1st subculture after that 10000 ppm salinity significantly depressed shootlet number per explant, shootlet length (cm). The number of leaves per shootlet and root length (cm) was decreased, while the root number per plantlet was significantly increased by increasing the levels of salinity.

For biochemical constituents of the shootlet, increasing the levels of salinity from zero to 10000 ppm significantly decreased the chlorophyll-a,-b, carotenoids and indoles, and increased the total soluble phenols and prolin. The SDS-PAGE protein profile analysis was carried out in the different concentrations of salt on *Solidago altissima* shootlet.

The survival percentage was 97-100% in all growing media which were used in this study.

Key words: *Solidago altissima, In vitro* propagation, salinity

INTRODUCTION

Solidago altissima (Golden rod) family Astraceae (Compositeae), is one of the most important perennial ornamental plants produces golden-yellow flowers in many minute heads. Some solidago species were recently introduced to Egypt for commercial production. They have a wide application as landscape plants, and as excellent cut flower for export.

Salinity is one of the major problems of agriculture in arid and semiarid regions. The accumulation of salts in soil may be attributed to high ground water table accompanied with poor drainage. Soil salinity has, therefore a great impact on decreasing yield potential of the cultivated crops. Crops yield start declining when EC of the soil solution goes above 4 ds m^{-1} (Sairam *et al.*, 2002)

Addition of salt to water lowers its osmotic potential, thereby decreasing its availability to root cells. Thus, salt stress exposes the plant to secondary osmotic stress, which implies that physiological responses, invoked by drought, can also be observed under salt stress (Sairam *et al.*, 2002).

Endogenous hormonal levels of plants were reported to undergo various and rapid changes under water stress and salinity (Shakirova, *et al.*, 2003; Azooz *et al.*, 2004).

The response of the plants to salt stress is results of accumulate and salts in plant tissues. The ability of plants to accumulate salts in their tissues is commonly known as "osmotic adjustment". This phenomenon is due to reduce of the water potential in the plant cells, which become low than in the external environmental, thus ensures flow of water into the plant.

Up till now, there are no literatures available on the salinity tolerance on *Salidago altissima* by *in vitro* culture

In resent years, the *in vitro* culture becomes one of the suitable techniques for developing the micro propagation program of high value ornamental species. To carry out trials in order to enhance the tolerance of *in vitro* produced *Solidago altissima* plantlets, as they are considered salinity susceptible plants. The effect of salinity on the micro propagation of several ornamental plants have been studied on the *Thuja orientalis* and *Adhatoda vasica* (Nofal *et al.*, 1983); *Acalypha macrophylla* and *Justica gendarus* (El-Shawakh, 1995); *Lemon grass* (Tawfik, 1986); *Cupressus sempervirens* (Shehata, 1992); *Lantana camara* (EL-Bagoury *et al.*, 1994) and *Cumacrops humillis* and *Phoenix canarienses* (Nofal *et al.*, 2001a)

Therefore, the present study was carried out to investigate the effect of various concentrations of sodium chloride (NaCl), calcium chloride ($CaCl_2$) and magnesium sulphate ($MgSo_4$) treatments on micropropagability and biochemical constituents of *Solidago altissima* Var "Tara" explants *in vitro*.

MATERIALS AND METHODS

This study have been carried out on the Tissue Culture and Germplasm Conservation Res. Lab., Hort. Res. Institute (ARC) Egypt, during the period from 2005 to 2006, in order to investigate the influence of different levels of salinity stress on the *in vitro* probability and biochemical constituents *Solidago altissima* Var "Tara" of explants (during two consecutive micro propagation stages of shooting and rooting).

Plant materials

The *in vitro* shootlet of *Solidago altissima* Var "Tara" (5-7cm high) were used in tissue culture as source of vegetative material. The shootlets were aseptically sectioned into explants (2-3 cm length) for use in salinity treatments.

Culture medium

The used MS-culture medium was the formulation of Murashig and Skoog (1962) enriched with (per liter) benzylaminopurin (BAP) (0.5 mg), sucrose

(30 g) and Agar (7 g). The medium was adjusted to pH 5.7 ±0.1, then paused at 25 ml in 200 ml capacity glass jars before autoclaving at 121°c and 1.2 kg/cm^2 for 15 min

Culture condition

The culture were incubated in growth chamber at 24 ± 1° under 16 hr photoperiod (day light flouresent tube) at 3 K lux.

Salinity stress treatments

The shootlets under investigation cultured in this medium were subjected to different levels of salinity treatments (0.0, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000 and 11000ppm). Salinity treatments consisted of mixture of sodium chloride (NaCl), calcium chloride ($CaCl_2$) and magnesium sulphate ($MgSo_4$) at ratio of 2:2:1 by weight, respectively. 12 treatments were done in this experiment.

***In vitro* propagation study**

One month after culturing, the formed shootlet were recaptured on fresh medium for three times. Therefore, in three consecutive shootlet proliferation cycles, twelve components of culture medium were done in 1st subculture and eleven components of culture medium in the 2nd, 3rd subcultures, rooting and acclimatization, twenty shootlet explants in five replicates for each treatment were used. After each subculture the following data were recorded: [survival percentage of explant, shootlet number per explants, shootlet length (cm) and number of leaves per shootlet].

Biochemical analysis

The shootlets which were obtained after the three subcultures were cut into small pieces and severed for pigments, indole and phenol analysis. For pigments determination, the ethanolic extractions were submitted to procedures to Saric *et al.*, (1967) to quantitative determine the endogenous chlorophyll-a&-b and carotenoids. For total indoles determination, the methods of Selim *et al.*, (1978). For total soluble phenols determination, the procedure of Daniel and George (1972) was used. Free proline concentration was measured calorimetrically in the extraction of fresh leaves using ninhydrin reagent according to Bates *et al.*, (1973). For protein, sodium dodecyl sulfat-polyacrylamide gel electrophoresis (SDS) was performed in 10% acrylamide slab gels following the system of Laemmli (1970) to identify by their protein profiles.

Rooting stage

In rooting, shootlet explants (3-4 cm length) resulting from the three subcultures were cultured in half- salt strength MS medium with different levels of salinity treatments (0.0, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000). Twenty-five shootlet in five replications were used for each treatment. At the end of the fourth week of culturing, the root number and root length (cm) were recorded.

Acclimatization stage

In the acclimatization trail, the rooted shootlet resulting from the different treatments were transferred to plastic pots (0.2 liter) containing peat moss plus washed sand at 1:1 (v/v) as growing media. The same salinity concentrations (0.0, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000) were used in irrigated the substrate and covered by transparent polyethylene bages in five vitroplants in five replicates for each treatment. The acclimatized vitroplants were kept in acclimatized glasshouse for four weeks after that the survival plants were recorded.

Statistical analysis

The layout of all experiments was a completely randomized design with one and two factors. The data were statically analyzed for testing differences between means using L.S.D. according to Steel and Torrie, (1980).

RESULTS AND DISCUSSION

1. Shooting stage behaviour

Survival percentage

Data in Table (1) showed a negative effect of salinity stress on survival percentages. Salinity concentrations more than 6000 ppm significantly decreased the survival percentage of the explants *in vitro*. This effect significantly increased with increasing the salinity levels. The lowest survival percentage zero was obtained with the highest salt concentration 11000 ppm. Using 10000 ppm salinity produced the lowest survival percentage 44.4%.

Table (1): Effect of salinity stress on survival percentage of *Solidago altissima* explants *in vitro*.

Treatments	Subculture 1	Subculture 2	Subculture 3	Mean B
Control	100.0	100.0	100.0	100.0
1000 ppm	100.0	100.0	100.0	100.0
2000 ppm	100.0	100.0	100.0	100.0
3000 ppm	100.0	100.0	100.0	100.0
4000 ppm	91.67	100.0	100.0	97.22
5000 ppm	83.33	91.67	100.0	91.67
6000 ppm	83.33	91.67	100.0	91.67
7000 ppm	75.00	75.00	83.33	77.78
8000 ppm	58.33	66.67	83.33	69.44
9000 ppm	41.67	50.00	58.33	50.00
10000 ppm	25.00	41.67	66.67	44.44
11000 ppm	00.00	00.00	00.0	00.00
Mean A	71.53	76.39	82.64	
LSD A		5.158		
B		10.32		
A×B		17.87		

The results suggested that salt concentrations from zero till 6000 ppm had no significant effect in the survival percentages of the explants. This means that salinity concentration in the micro propagation medium up to 6000 ppm can be used without any significant reduction in the survival percentages.

The effect of salinity concentrations on relation to the number of subcultures indicated that the lowest survival percentage (71.53%) in the 1st subculture increased gradually in the 2nd (76.39%) and 3rd subculture, which gave the highest percentage (82.64%). This means that the explants become more tolerance by increasing the number of subcultures.

The interaction between salinity concentration and the number of subcultures revealed that in the 1st subculture using zero to 3000 ppm had no significant effect on the survival percentages. However, increasing the concentrations more than 3000 ppm caused a highly significant decreased in this character. In case of the 2nd and 3rd subcultures up to 4000 and 6000 ppm salinity concentration had no significantly effect on the percentage of survival respectively. In this respect Mohamed (1996) on *Casuariena glauca,* concluded that the addition of chloride salts to the soil reduced the survival percentage

Shootlet number per explant

Data in Table (2) revealed that the different levels of salinity stress had a highly significant effect on decreasing the shootlet number per explants. The highest number of shootlets was recorded in the control treatment (11.17 shootlets /explant) compared to 10000 ppm treatment. Which the lowest number was (1.08 shootlet/explant). The number of subcultures significantly affected the number of shootlets/explant. Increasing number of subcultures increased the number of shootlets/explant for the 1st, the 2nd and the 3rd subcultures, which gave 2.44, 2.67 and 3.41 shootlets/explant, respectively. For the effect of different levels of salinity with the number of subcultures, the highest number of shootlet/explant was obtained in the 3rd subculture compared with control (12.0 shootlets/explant), while the lowest number of shootlets/explant was observed on the 1st and the 2nd subcultures with 10000 ppm salinity, which gave 1.0 shootlets/explant. In this respect, Sayed (2006) on *Arecastrum romanzoffianum* found that, the highest levels of salinity depressed the shootlet number.

Shootlet length (cm)

The different levels of salinity had a highly significant effect on decreasing the shootlet length as shown in Table (2). The shootlet length varied from 0.73 to 2.64 cm. The longest shootlet was obtained in the control treatment; however, the shortest one resulted from using 10000 ppm salinity concentrations.

The data indicated that in the 1st subculture significantly decreased in shootlet length (1.186 cm) was observed compared to the 2nd and 3rd subcultures (1.546 and 1.71 cm, respectively). The longest shootlet was observed in the 3rd subculture. This means that increasing the subcultures caused an increasing in the salinity stress and increasing the plant tissues tolerance.

The interaction between the levels of salinity and the number of subcultures revealed that the longest shootlet (3.01 cm) resulted from the control treatment, however, the lowest values (0.57 cm) obtained from explants treated with the highest concentration (10000 ppm) in the first subculture. These results are in agreement with other workers as Downton and Loveys (1991); Gaser (1992); Ismail (1996) all worked on grapevine and Nofal *et al.*, (2001a and b) on some ornamental palm trees

Table (2): Effect of salinity stress on shooting stage of *Solidago altissima* explants *in vitro*.

Treatments	Shootlet number/explant			
	Sub. 1	Sub. 2	Sub. 3	Mean B
Control	10.75	10.75	12.00	11.17
1000 ppm	3.42	3.67	4.17	3.75
2000 ppm	2.58	3.33	3.83	3.25
3000 ppm	2.08	2.58	3.17	2.61
4000 ppm	1.33	1.58	2.83	1.92
5000 ppm	1.42	1.58	2.58	1.86
6000 ppm	1.08	1.33	2.42	1.61
7000 ppm	1.08	1.25	2.00	1.44
8000 ppm	1.08	1.17	1.83	1.36
9000 ppm	1.00	1.08	1.42	1.17
10000 ppm	1.00	1.00	1.25	1.08
Mean A	2.439	1.667	3.409	
LSD A	0.2868			
B	0.5491			
A×B	0.9511			
	Shootlet length (cm)			
Control	2.18	2.72	3.01	2.64
1000 ppm	1.42	2.02	2.23	1.89
2000 ppm	1.46	2.27	2.44	2.06
3000 ppm	1.37	1.67	1.40	1.48
4000 ppm	1.04	1.77	2.04	1.62
5000 ppm	1.11	1.43	1.84	1.66
6000 ppm	1.15	1.23	1.38	1.26
7000 ppm	0.98	1.11	1.25	1.11
8000 ppm	1.14	1.26	1.36	1.25
9000 ppm	0.63	0.81	0.94	0.79
10000 ppm	0.57	0.71	0.92	0.73
Mean A	1.186	1.546	1.710	
LSD A	0.1930			
B	0.3860			
A×B	0.6686			
	Leaves umber/shootlet			
Control	9.02	9.09	9.85	9.32
1000 ppm	7.12	9.19	9.50	8.60
2000 ppm	8.85	9.99	10.37	9.74
3000 ppm	8.72	8.10	8.45	8.42
4000 ppm	8.01	8.92	9.54	8.83
5000 ppm	7.96	8.60	8.92	8.49
6000 ppm	8.08	8.15	8.42	8.22
7000 ppm	8.11	7.29	7.58	7.66
8000 ppm	8.11	7.63	7.50	7.75
9000 ppm	5.33	6.71	6.79	6.28
10000 ppm	4.67	7.54	7.46	6.56
Mean A	7.634	8.292	8.580	
LSD A	0.5723			
B	1.096			
A×B	1.898			

Number of leaves per shootlet

Table (2) revealed that, the number of leaves /shootlet significantly decreased by subjecting to salinity stress and number of subculture. For levels of salinity stress, (2000 ppm) salinity produced the greatest number of leaves/shootlet (9.74), while adding (10000ppm) decreased the number of leaves/shootlet to the lowest number (6.56 leaves/shootlet). The highest number of leaves/shootlet (8.58) was recorded in the 3rd subculture compare with the 1st subculture, which gave (7.63 leaves/shootlet). The effect of levels of salinity stress and the number of subcultures showed that the greatest number of leaves/shootlet was obtained in (3000ppm) in the 3rd subculture, which gave (10.37 leaves/shootlet). The (10000 ppm) salinity stress in the 1st subculture recorded the smallest number of leaves/shootlet (4.67). The obtained results clarify that salinity stress brought about a depression in leaves number. These results could be attributed to the effect of salinity on reducing the synthesis of DNA, RNA and protein in many plants which it might be lead to disturbance in metabolic activities. Hegazi (1974) and Kalil (1986) on *Ricinus communis* and *Hyoscamun muticus*.

2. Biochemical constituents of shootlet explants

Chlorophylls and carotenoids contents (mg/100g F.W.)

The data in Table (3) revealed that the different salinity concentrations showed a highly significant effect on decreased chlorophyll-a content except 2000 ppm concentration which gave the same value as the control. In general, the chlorophyll-a content varied from 99.69 to 31.99 mg/100g F.W. The lowest content of chlorophyll-a was found by using 9000 ppm salinity concentration.

Table (3): Effect of salinity stress on biochemical constituents of *Solidago altissima* explants *in vitro*.

Treatments	Pigments mg/100g F.W.			Indol mg/100g F.W.	Phenole mg/100g F.W.	Prolin mg/100g F.W.
	Chl. A	Chl. B	Caro.			
Control	99.69	52.40	127.0	670.2	208.7	0.187
1000 ppm	75.46	36.24	96.44	401.0	654.1	0.197
2000 ppm	95.68	57.61	111.3	600.1	597.5	0.320
3000 ppm	48.06	49.98	80.70	560.0	671.5	0.387
4000 ppm	54.44	46.97	62.43	410.7	823.4	0.547
5000 ppm	46.51	32.26	73.96	398.3	470.1	1.678
6000 ppm	89.72	87.93	98.86	407.8	420.2	0.940
7000 ppm	51.47	61.03	57.50	339.7	1031.0	0.972
8000 ppm	78.54	112.5	84.77	603.9	10.57.0	0.797
9000 ppm	31.99	51.31	72.71	280.3	1065.0	0.677
10000 ppm	49.26	45.28	61.23	221.3	1754.0	3.337
LSD	4.331	10.94	6.862	224.5	99.77	0.9784

In case of chlorophyll-b content the data showed another trend. The highest value 112.5 mg/100g F.W. was resulted in case of using 8000 ppm salinity concentration. Whereas, the lowest value 36.24 mg/100g F.W. was found in 1000 ppm concentration.

The carotenoids content showed the same trend as chlorophyll-a content. The highest value 127.0 mg/100g F.W. was obtained in the control treatment whereas, the lowest value 61.23 mg/100g F.W. was resulted in case of using the highest salinity concentration 10000 ppm. This data are harmony with Bolu and Polle (2004) on *Populus*.

Total indoles

The data in Table (3) indicated that using salinity concentrations 2000, 3000 or 8000 ppm had no significant effect on total indoles contents compared to the control. Whereas, the other concentrations of salinity had a highly significant effect on decreasing the total indoles contents in the shootlet. The highest value (670.2 mg/100g F.W.) were resulted from the control treatment whereas, the lowest value (221.3 mg/100g F.W.) was found in case of using 10000 ppm salinity concentration. The reduction in indoles as a result of salinity stress may be ascribed to the increase of IAA-oxidase under stress conditions. These results are consistent with other workers such as Abd El-Ghany (1990); Downton and Loveys (1991); Gaser (1992); Ismail (1996) and Nofal *et al.*, (2001 a and b) on grapevine rootstocks and ornamental palm.

Total soluble phenols

Table (3) showed the promotive effect of salinity stress on total soluble phenols accumulation while exhibite favorable increase in harmony with the elevation of salinity levels with significant difference. The immensity of increase of phenols was fulfilled with higher value in shootlet (1754.0 mg/100g F.W.) at the highest level of salinity 10000 ppm. The least phenols concentration (208.7 mg/100g F.W.) took place at control. Increasing salinity levels were accompanied by a gradual increase in the plant concentration of total soluble phenols. Moreover, it is quite clear that there is a reversible relation between phenols compounds accumulation and indoles reduction under salinity stress conditions. Several workers (Van Sumere *et al.*, 1975; Popovici and Rezink, 1976 and Hanafy, 1991 a & b and 1996 on *Spinacia oleraceae*, L.) postulated that phenolic compounds are capable of inhibiting ATP synthesis in mitochondria, uncoupling respiration, affect polar transport of auxins, inhibiting enzyme activity, antagonizing plant hormones biosynthesis and inhibiting ions absorption.

Proline concentration

Data in Table (3) revealed that salinity stress had a significant increase in proline concentration. The highest proline value (3.337 mg/100g F.W.) occurred at salinity level (10000 ppm). While the lowest values (0.1867 and 0.1967 mg/100g F.W.) were detected at control and 1000 ppm salinity respectively. These results may be referred to gene controls proline accumulation, called Osmotic Tolerance Gene, which governs the production of a class of molecules such as beaten and proline that protect the cell and its constituents against dehydration. Proline is considered as a cytoplasm protective osmolyte necessary for adaptation to stress (Rudulier *et al.*, 1984), Moeover, Roy *et al.*, (1992) stated that proline accumulation in response to NaCl could be attributed to both an increase in D-pyrrolin- 5carboxylate reductase (PCR) and a decrease in proline dehydrogenase (PDH) activity. In addition, Lutts *et al.*, (1996) and El-Adawe (2005) on *Pheonix dactylifera* conducted that salt resistant cultivars accumulate lower amounts of free proline than salt sensitive ones.

Protein fractions

The SDS-PAGE protein patterns as biochemical markers to distinguish the differences between concentrations of salt of *in vitro* shootlet illustrated in Fig (1). However, the most significant differences are found in the absence of two bands with molecular weights 114.0 and 66.0 KDa (Kilo Dalton) except in 4000 ppm in 114.0 KDa and 4000 and 5000 ppm in 66.0 KDa. In 33.8 KDa control and low concentration of salt to 3000 ppm lead to absence of bands. On the other hand, in 20.1 KDa the increasing concentrations of salt up to 2000 ppm lead to absence of bands. These results are in harmony with Ben-Hayyim *et al.* (1989) indicated that the degree of a change in the level of a gavin protein does not necessarily indicated its importance in adaptation to stress conditions. The level of a protein product of a regulatory gen is expected to be low, but it may still be the key protein in the process of adaptation.

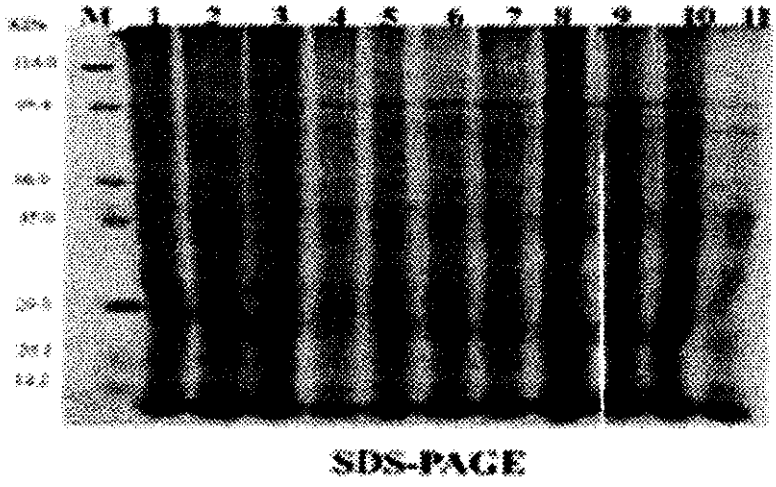

Fig (1): Patterns of SDS- PAGE electrophoretic protein *Solidago altissima* after salinity stress.

1-control	2- 1000ppm	3- 2000 ppm	4- 3000 ppm
5- 4000 ppm	6- 5000 ppm	7- 6000 ppm	8- 7000 ppm
9 8000 ppm	10- 9000 ppm	11- 10000 ppm	

Rooting behaviour

Data in Table (4) showed that salinity stress significantly influenced root number per plantlet and root length (cm). The root number was increased by increasing salinity levels from control to 10000 ppm, which gave (3.14 to 4.92 root/plantlet, respectively). While the highest salinity level 10000 ppm brought the shortest length of roots (1.30 cm) compared to control (3.33 cm). These results were in agreement with those found by Ramolia *et al.*, (2004) on *Acacia catechu* who ascribed the production of young roots and death of old roots were found to continuous and plant apparently use this processes an avoidance mechanism to remove excess ions and delay onset of ion accumulation in this tissue, this phenomenon, designated (fin root turnover) is of important to the mechanism of salt tolerance.

Acclimatization (survival percentage)

The different salinity stressed levels had no significant effect on the survival percentage in Table (4). This means that all levels of salinity stress on rooting stage produced plantlet successfully in acclimatization and produced high survival percentages ranging from 100-97%.

Table (4): Effect of salinity stress rooting behaviour and acclimatization plantlet of *Solidago altissima* explants *in vitro*.

Treatments	Rooting behaviour		Acclimatization stage
	Root number	Root length (cm)	Survival %
Control	3.14	3.33	100
1000 ppm	3.14	3.00	100
2000 ppm	2.75	2.83	97
3000 ppm	2.92	2.83	97
4000 ppm	2.75	2.70	98
5000 ppm	3.83	2.77	97
6000 ppm	3.42	2.17	98
7000 ppm	3.83	1.97	99
8000 ppm	4.67	1.43	97
9000 ppm	4.92	1.57	98
10000 ppm	4.92	1.30	97
LSD	1.591	0.727	NS

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دراسات على مدى تحمل السوليداجو التيزما للملوحة فى مزارع الانسجة

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اجرى هذا البحث بمعمل زراعة الانسجة وحفظ الاصول الوراثية بمعهد بحوث البساتين- مركز البحوث الزراعية خلال الاعوام ٢٠٠٥ و ٢٠٠٦. ويهدف البحث الى دراسة تأثير التركيزات المختلف من الملوحة على سلوك المراحل المتتابعة للاكثار الدقيق لنبات السوليداجو التيزما *Solidago altissima*.

اظهر التحليل الاحصائى للنتائج انة فى مرحلة التكشف: ادى استخدام تركيز ١١٠٠٠ جزء فى المليون من الاملاح الى موت كل الاجزاء النباتية فىْ النقلة الاوّلى. كما ان استخدام ١٠٠٠٠ جزء فى المليون من الاملاح ادى الى تثبيط فى كل من عدد النبيتات المتكونة لكل جزء نباتى، طول النبيتات (سم)، عدد الاوراق لكل نبيتة وكذلك طول الجذور (سم) بينما ادى الى زيادة فى عدد الجذور المتكونة لكل نبتة.

زيادة تركيز الاملاح من الكنترول (بدون املاح) الى ١٠٠٠٠ جزء فى المليون الاملاح ادى الى نقص فى الكلورفيل أ، ب و الكاروتين وكذلك الاندولات بينما ادى الى زيادة فى الفينولات الكلية الزائبة وكذلك البرولين.

اقلمة النبيتات الناتجة من التركيزات المختلفة من الملوحة من (كنترول الى ١٠٠٠٠ جزء فى المليون) اعطت نسبة نجاح من ٩٧-١٠٠%.