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An Evaluation of Effect of Salt Stress on Callus Induction in Different Potato Cultivars

[1]*Setareh Forooghian and* [2]*Shahin Esfarayeni*

[1]Agriculture and Plant Breeding, Islamic Azad University of Borujerd Branch, Borujerd, Iran
[2]Agriculture of Islamic Azad University of Borujerd Branch, Borujerd, Iran

Abstract: In this study, callus induction of three potato (*Solanum tuberosum* L.) cultivars of sante, Savalan and Agria under salt stress were studied. This experiment was carried out in the completely randomized factorial design with 15 treatments and 3 replications. After amplification of these cultivars' seedlings using in-vitro amplification method they were cultured for callus induction on MS medium containing 5 mg/L 2, 4-D and 2 mg/L of kinetin. Then the produced calluses were put under salt stress from different concentrations of NaCl (0, 5, 75 and 100 Millimolar). In this experiment wet weight, dry weight, relative water content of callus and dry weight to wet weight ratio of callus were studied. The results showed that meaningful differences existed among cultivars of all studied characters. While among different salt stresses levels, there was meaningful differences between characters of wet and dry weight of callus. By increasing the salt stress (NaCl concentration) in different studied cultivars, the dry and wet weight of callus meaningfully decreased and smaller calluses with darker colors were produced. Also the Agria cultivar showed the maximum tolerance to salt stress compared to other cultivars. So that in this cultivar, the decrease rate of dry weight of callus in the highest concentration (100 Millimolar NaCl) compared to the control group was 39.96%.

Key words: Potato · Tissue culture · Callus induction · Salinity · Sodium chloride

INTRODUCTION

Anyway, cellular-level research about ions effects (Na, Cl, …), herbicides, pesticides and toxins resulted from pathogens are useful. In these studies one can use different strategies. One is to choose cells that can better tolerate these compounds and then choosing the more tolerable cells. These cells may grow by increasing the value of the agent of choice in the medium. In some studies the choice is done using a step by step method and the agent of choice in the medium in every reaction is gradually increased. If one can regenerate plants in this way, maybe selection in cellular-level would be able to increase the resistance of the whole plant to the specific stress. Using this method one can choose better cells related to different genotypes considering resistance to a specific metal or salt and one can investigate the amount of resistance [1, 2] investigated the highest correlation between resistance to salt and dry substance if the plant has been put against the salt stress of the environment for a long time. Using short term experiments with seedlings was fast but unsuccessful, because measuring the growth for a short time is only a selection for resistance to low water potential and is not related to the specific effects of the salt. Accomplished investigations have showed that among different agricultural species and among different cultivars of species from perspective of resistance to the salinity there are a lot of differences [3]. Potato is one of the plants that most of the in-vitro cultivation methods have been done on it. In in-vitro cultivation of this plant, tuber explants, branch, meristem, anther and pollen has been used and in some cases protoplast and mesophile for plant regeneration has been used [4]. Nowadays, plant tissue culture methods are a powerful tool for studying the problems related to botany. Increasing the plants resistance in stress conditions for economical performance production is one of the most important reform and eugenics goals. The belief is that using tissue culture can be complementary to common eugenic methods for increasing the resistance of plants especially resistance against salinity. Choosing plants that are resistant to living and non-living stresses can be

Corresponding Author: Setareh Forooghian, Islamic Azad University of Borujerd Branch, Borujerd-Iran.
Tel: +98-5115226516, Mob: +98-9153316366.

accomplished in the tissue and the cell as well and in the tissue culture method by using isolated cells, calluses and differentiated tissues and or resulted seedlings the ability to choose the variables that are resistant to a selectivity factor do exist. One can consider salinity as a general concept that expresses the presence of various mixtures of salts of the earth [5]. The results of in-vitro experiments of potato showed that the concentration of salt would directly interfere in the growth process and would prevent osmotic process and translocation in plants [6]. Generally, salinity has a meaningful effect on all of the characteristics of most of plants [7]. Adaption to salinity is proportional to tolerability and resistance of plants [8]. In salinity conditions the plant will respectively develop mild to severe yellow leaf and potassium and calcium concentrations will decrease [9]. Growth properties in lab conditions have been evaluated by some researchers and corresponding answers related to applying salt stress with a different behavior for every specie or cultivar have been reported [6]. Reaction to salinity in transgenic potato genotypes in lab conditions has been also investigated recently [10]. According to some of the researches, the highest salinity amount (100 Millimolar NaCl) would completely prevent the growth of tubers in all of the cultivars but stolons would continue growing [7, 11] investigated resistance to salinity at vegetative growth stage in some of the cultivars of potato in in-vitro conditions and concluded that salinity had a meaningful decrease on all the characteristics except the dry weight of root and the surface area of leaf. In this report considering these characteristics, Agria cultivars compared to Sante and Savalan cultivars in non-salinity conditions were superior and had a low resistance to salinity. [12] studied the effect of salinity on callus induction of potato and reported that salinity would decrease calluses growth. [7-9, 13] reported that with increase of NaCl levels, stolons growth would not be limited but the tubers and callus growth would be limited. [14, 15] investigated the salt stress on potato and reported that commercial cultivars (Agria cultivars) have a low resistance to salinity. Most of the reports express that salinity would decrease the growth and would produce dry substance in plants [16-19]. Without doubt using modified plant cultivars with higher quantitative and qualitative performance and compatible to limited agricultural conditions such as water deficit, heat, cold, salinity, soil nutrient deficiency, diseases and pests are the main key for increase in efficiency rate of basic resources of soil and water and the influence rate of other production inputs. Considering that water deficiency and salinity of soil and water resources would limit the growth of plants and production and on the other hand the extent and distribution of salty soils in our country is very widespread and also salt stress would cause decrease in potato product performance, so in this study callus induction of different potato cultivars under salt stress with the use of tissue culture technique was studied and evaluated.

MATERIALS AND METHODS

This research was carried out in the biotechnology laboratory of agricultural and natural resources research center of Khorasan Razavi. In this study different levels of NaCl (0, 25, 50, 75, 100 Millimolar) on callus induction of three potato cultivars of Agria, Sante and Savalan were studied. This experiment was carried out in the completely randomized factorial design with 3 replications. Every replication contained 10 test tubes and in every test tube were one callus. The medium that was used in this experiment was modified MS medium containing 2, 4-D and kinetin hormones with the amount of 5 and 2 mg/liter respectively. Also 30 gr/liter sucrose was added to the mentioned environment. Then the solution PH was adjusted to 5.7 the amount of used Agar is 6.7 gr/liter. In order to sterilize the medium, autoclaving conditions of 121°C and pressure of 0.5 bars for 15 minutes was used. After preparation of the medium, explants are cultivated in sterile grow room conditions and under laminar flow cabinet. First sterile seedlings must be divided into explants having one seedling with the length of 5 millimeters in sterile conditions and then for callus production they would be placed inside a medium containing 2, 4-D and kinetin hormones with the amount of 5, 2 milligrams respectively. After cultivation, for callus induction explants are transferred to grow room under condition of 16 hours of lighting and 8 hours of darkness with light intensity of 5000 lux and the temperature of 25±2°C. Produced calluses of studied cultivars are placed under salt stress resulted from different concentrations of NaCl (0, 25, 50, 75, 100 Millimolar). For applying mentioned salinity treatments 0, 1.461, 2.922, 4.383, 5.844 gr/ liter of sodium chloride were added to the medium respectively. After four weeks characteristics such as wet and dry weight of callus, relative water content of callus and dry weight to wet weight ratio of callus were measured. Relative water content of callus was calculated using the formula below [20]:

Relative water content of callus = (Wet weight of callus – Dry weight of callus) ÷ Wet weight of callus × 100

Statistical calculations and plotting the graphs were done using Excel and SAS software respectively. Median comparison was done using MSTAT-C software and by using LSD test with probability level of 5 percent.

RESULTS

Variance analysis results show that the effects of cultivars on all the studied characteristics at probability level of 1 percent are meaningful. The effect of different salinity levels for all the studied characteristics, except for the water content of callus and the wet to dry weight of callus at 1 percent level was meaningful. Also additionally, the effects of contrasting cultivars at different salinity levels for all the studied characteristics and except for the water content of callus and the wet to dry weight of callus at 1 percent level were meaningful (Table 1).

Comparing the results of different levels of salinity on wet weight character of callus shows that the highest wet weight of callus is related to the control group treatment with an average of 697.11 milligram and the lowest wet weight of callus was related to the 75 Millimolar concentration of NaCl with an average of 111.63 milligram and on the other hand among concentrations of 50, 75 and 100 Millimolar of NaCl considering the wet weight character of callus there was no meaningful differences. Also the highest dry weight of callus is related to control group treatment with an average of 48.27 milligrams and the lowest dry weight of callus was related to the 100 Millimolar concentration of NaCl with an average of 9.96 milligrams. Additionally, the highest water content of callus was related to the treatment of 25 Millimolar with an average of 91.83 percent and the lowest amount of this character was related to control group treatment with an average of 89.02 percent. Also, the highest dry to wet weight ratio was related to the treatment of 75 Millimolar NaCl with an average of 9.29 percent and the lowest dry to wet weight ratio of callus was related to the control group treatment with an average of 7.64 percent (Table 2).

Comparing the cultivar effect on wet weight of callus shows that the highest wet weight of callus was related to Savalan cultivar with an average of 472 milligram and the lowest one was related to Agria cultivar with an average of 70.72 milligrams. The highest dry weight of callus was related to Savalan cultivar with an average of 40.18 milligrams and the lowest one was related to Agria cultivar with an average of 7.02 milligrams. Also among Sante and Agria cultivars considering dry weight character of callus there was no meaningful differences. Additionally, the highest water content of callus was related to Agria cultivar with an average of 93.69 percent and the lowest water content of callus was related to Agria cultivar with an average of 87.83 percent. Also the

Table 1: Variance analysis results of salt stress effects on different potato calluses

		Mean-square			
Sources of changes	Degrees of freedom	Wet weight of callus	Dry weight of callus	Water content of callus	Dry weight to wet weight ratio of callus
Cultivar	2	614161.783**	4781.710**	115.112**	67.227**
NaCl concentration	4	569504.916**	2399.844**	4.61ns	3.927ns
NaCl concentration * Cultivar	8	134122.965**	693.876**	14.028ns	1.668ns
Experimental error	30	8986.450	79.875	11.81	2.261

** Meaningful at 1% level and non-meaningful ns

Table 2: Comparing the average of different levels of salinity effect on the studied character

NaCl concentration (Millimolar)	Wet weight of callus (milligram)	Dry weight of callus (milligram)	Water content of callus (percent)	Dry to wet weight ratio of callus (percent)
0	697.11a	48.28a	89.02a	7.64b
25	231.61b	18.83b	91.83a	8.23ab
50	126.07c	11.39bc	91.37a	8.49ab
75	111.63c	10.46bc	90.65a	9.29a
100	113.65c	9.96c	91.41a	8.40ab
LSD	91.26	8.60	3.31	1.45

In each column, figures with shared letters have no meaningful differences with each other (LSD and α=5%)

Table 3: Comparing the average of the main effect of cultivar on the studied characters

Cultivars	Wet weight of callus (milligram)	Dry weight of callus (milligram)	Water content of callus (percent)	Dry to wet weight ratio of callus (percent)
Sante	225.92b	12.14b	93.69a	6.24b
Savalan	472a	40.18a	91.04b	8.97a
Agria	70.72c	70.2b	87.83c	10.02
LSD	70.69	6.66	2.56	1.12

In each column, figures with shared letters have no meaningful differences with each other (LSD and α=5%)

Table 4: Comparing the average of cultivar contrasting effects * salinity on wet and dry weight of callus

Cultivars	NaCl concentration (Millimolar)	Wet weight of callus (milligram)	Dry weight of callus (milligram)
Sante	0	828.7b	41b
	25	120.5defg	7.6def
	50	76899efg	5f
	75	61.22fg	4.5f
	100	42.33g	2.5f
Savalan	0	1166.61a	94.6a
	25	501.5c	41.17b
	50	231.3de	22.8c
	75	218def	20.8cde
	100	242.6d	21.5cd
Agria	0	96.6defg	9.2cdef
	25	75.83efg	7.6def
	50	70fg	6.3ef
	75	55.67g	6ef
	100	56.06g	5.8f
	LSD	158.07	14.90

In each column, figures with shared letters have no meaningful differences with each other (LSD and α=5%)

highest dry to wet weight ratio of callus was related to Agria cultivar with an average of 10.02 percent and the lowest dry to wet weight ratio of callus was related to Sante cultivar with an average of 6.24 percent (Table 3).

Comparing the average of contrasting cultivar effects on different salinity levels shows that the highest wet weight of callus was related to the Savalan cultivar in the control group treatment (without NaCl) with an average of 1166.61 milligrams and the lowest one was related to 100 Millimolar Sante cultivar with an average of 42.33 milligrams. The highest dry weight of callus was related to Savalan cultivar in control group treatment with an average of 94.6 milligrams and the lowest dry weight of callus was related to sante cultivar in 100 Millimolar solution with an average of 2.5 milligrams (Table 4).

DISCUSSION

The results of salt stress effect experiment on callus induction of studied cultivars showed that among studied cultivars and also different concentrations of NaCl considering the wet and dry weight of callus there was meaningful differences ($P \leq 0.01$). Additionally with increase in salt stress (NaCl concentration) the wet and dry weight of callus were decreased meaningfully in the studied cultivars. But there was no meaningful difference in water content of callus with increase in salt stress. Also the Agria cultivar calluses had the highest resistance to salinity compared to other studied cultivars. So that the decrease rate of dry weight of callus in the highest concentration of NaCl (100 Millimolar) compared to the control group (without stress) in cultivars of Sante, Savalan and Agria was 93.6, 77.27 and 36.96 percent. It should be noted that the rate of resistance to stress level in the callus of Savalan cultivar was at average level (semi sensitive) and callus of Sante cultivar is sensitive to salinity as well. [11] with investigating resistance to salinity at vegetative growth stage in some of the cultivars of potato in in-vitro conditions showed that salinity had a meaningful decrease on all the characteristics except the dry weight of root and the surface area of leaf and the commercial cultivar of Agria compared to non-commercial cultivars of Sante and Savalan in non-salinity conditions were superior considering these characteristics and had a higher resistance to salinity. [12] studied the effect of salinity on callus induction of potato and reported that salinity would decrease calluses growth. Some researchers reported that

with increase of NaCl levels, stolons growth would not be limited but the tubers and callus growth would be limited [7-9, 21, 13]. Growth characteristics in some of the potato cultivars has been evaluated by some researchers in lab conditions and corresponding answers for applying salt stress has been reported with a different behavior for every cultivar [6]. According to some of the researches, the highest amount of salinity level (100 Millimolar NaCl) would completely prevent the growth of tubers in all of the cultivars, but stolons would continue growing [7]. Results showed that as NaCl concentration is increased, because of decrease in cell water, cell division would decrease and less starch is produced so smaller calluses with darker colors would be produced. Generally, the results of this study showed that the calluses of Sante, Savalan and Agria cultivars in terms of resistance to salinity are sensitive, semi-sensitive and resistant respectively.

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