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Research Article

Salt tolerance of genetic modified potato (*Solanum tuberosum*) cv. Agria by expression of a bacterial mtlD gene

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Abstract. Water and salinity stresses are the major reasons to yield decreasing in the world. Potato is the world's main tuber crops of the Solanaceae family which is one of the most economically important annual vegetable crop. The goal of this investigation was creation genetic modified potato cv. Agria with more tolerance to salinity stress and evaluating GMO potato properties. To create transgenic potato plant, mtlD gene (mannitol-1-phosphate dehydrogenase, E.C.1.1.1.17) was expressed to potato cv. Agria plant by using *Agrobacterium tumefaciens*. Transgenic potato was produced by transforming of mtlD gene to potato plant cv. Agria. Existence of recombinant gene in transgenic plants was approved by two ways 1. Polymerase Chain Reaction technique. 2. Measurement of physiological parameters. The transgenic potatoes and non-transgenic potatoes lines exhibited the different amounts of tolerance to salinity stress because in the transgenic lines mannitol accumulates that increased osmotic pressure in salinity stress. The salt tolerance of transgenic potato cv. Agria (+mtlD) was recorded higher than that of non-transgenic potato cv. Agria (-mtlD). Osmotic pressure in this transgenic potato plant was increased by accumulating of mannitol and existence of mannitol in potato plant approved that the mtlD gene was successfully expressed to potato cv. Agria.

Key Words: transgenic potato, mtlD gene, salinity stress.

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Introduction

Potato (*Solanum tuberosum*) is a tuber plant from Solanaceae family which is grown all over the world. The potato plant was first grown and eaten in the Andes Mountains of South America then it was brought to Europe and other countries (Blas & Petrescu 2009). It is the most important crop after rice, wheat and corn (Vreugdenhil & Bradshaw 2007), and the year 2008 was named as international year of potato by United Nation (Theisen 2008). The potato tuber (of a medium size, 150 g) contains vitamin C (27 mg, 45% of the daily value), 620 mg potassium (18% of the daily value), 0.2 mg vitamin B6 (10% of the daily value), thiamin, riboflavin, niacin, phosphorus, iron and zinc. It was reported by the United Nations - FAO that the world production of potatoes in 2009 was 330 million tones. Two thirds of the global production is eaten directly by humans and the rest is used to feed the animals or produce starch (Vreugdenhil & Bradshaw 2007). One the major branch of genetic engineering is genetic modified organisms (GMO). Although this term (GMO) is not the most representative for genetically engineered organisms, GMO was defined as an organism which his genetic material has been altered using genetic engineering techniques (Bhatnagar *et al* 2007). These techniques were initially named recombinant DNA technologies, and use DNA molecules from different sources that are combined into one chromosome to create a new organism with modified genetic material. Genetic modification includes the insertion of a wanted gene or genes to genetic material of a cell (Bahieldin *et al* 2007). GMO is used in biological and medical research production of pharmaceutical drugs, gene therapy and agriculture. Many GMO organisms that were created include plants, fishes, bollworms, mosquitoes, fruit flies and microbes (Katiyar-Agarwal *et al* 1999).

Abiotic stresses have unfavorable effect on growth and productivity of plants and those cause a series of morphological, physiological, biochemical and molecular changes in plants. Drought, temperature extremes and saline soils are the most common abiotic stresses. Approximately 22% of the agricultural lands are saline, and regions under drought are increasing and it was forecasted the areas under salinity and water stress will be increased (Burk *et al* 2006). When a crop is exposed to abiotic stress, a number of genes are turned on, resulting in increased amount of some metabolites and proteins. Water and salinity stresses are main abiotic stresses which limit plant productivity and growth. One way of increasing yield in stress condition is use of genetic engineering for improving plant tolerance to water and salinity stresses (Abebe *et al* 2003). Horsch *et al*, in 1985, for first time introduced general method for transferring

genes into plant. They have developed an approach for the transformation that integrates the gene - transfer capability of *A. tumefaciens* with the simple and general regeneration capability of leaf explants. This method is more rapid and simple than previous methods (Horsch *et al* 1985). Tarczynski *et al*, in 1992, studied the expression of a bacterial mtlD gene in transgenic tobacco that leads to production and accumulation of mannitol. Mannitol concentration exceeded 6 µmol/g (fresh weight) in the leaves and in the roots of some transformants whereas this sugar alcohol was not detected in these organs of wild-type tobacco plants or untransformed tobacco plants (Tarczynski *et al* 1992). Tarczynski *et al* studied on the effect of mannitol which has produced in transgenic tobacco, on stress protection of tobacco plant. In this research was detected that the accumulation of sugar alcohols and other low molecular weight metabolites such as proline and glycine – betaine is a response that may protect against environmental stress. Growth of plant from control and mannitol-containing lines in the absence and presence of added sodium chloride was analyzed. Plants containing mannitol had an increased ability to tolerate high salinity (Tarczynski *et al* 1993). Phar & Stoop, in 1996, investigated the effect of excess macronutrients in the root environment on mannitol and sucrose metabolism in celery (*Apium graveolens* L. var dulce). Celery is one of plants that normally produce mannitol in its organs which increased ability to tolerate environmental stress such as salinity and water stress (Stoop & Phar 1996). Thomas *et al* transformed gene mtlD which encodes mannitol-1-phosphate dehydrogenase (E.C.1.1.1.17) into *Arabidopsis thaliana*. MtlD-transformant accumulated mannitol, a sugar alcohol that is not normally found in *Arabidopsis thaliana*. When mtlD-expressing seeds and control seeds were imbibed with solutions containing NaCl (range 0–400 mol/m^3), transgenic seeds containing mannitol germinated in medium supplemented with up to 400 mol/cm^3 NaCl, while control seeds ceased germination at 100 mol/m NaCl (Thomas *et al* 1995). Chakraborty *et al* (2000) increased nutritive value of transgenic potato by expressing a non-allergenic seed albumin gene from *Amaranthus hypochondriacus*. Improvement of nutritive value of crop plants, in particular the amino acid composition, has been a major long-term goal of plant breeding programs. They reported earlier the cloning of the seed albumin gene ama1 from *Amaranthus hypochondriacus*. The ama1 protein is non-allergenic in nature and is rich in all essential amino acids, and the composition corresponds well with the World Health Organization standards for optimal human nutrition. In this research to improve the nutritional value of potato, the ama1 coding sequence was successfully introduced and expressed in tuber-specific and constitutive manner. There was a noticeable increase in the growth and production of tubers in transgenic populations and also of the total protein content with an increase in most essential amino acids. The expressed protein was localized in the cytoplasm as well as in the vacuole of transgenic tubers. Thus they have been able to use a seed albumin gene with a well-balanced amino acid composition as a donor protein to develop a transgenic crop plan (Chakraborty *et al* 2000). Kondrak and coworkers (2011) studied on transcriptome analysis of potato leaves expressing the trehalose-6-phosphate synthase 1 gene of yeast. The trehalose-6-phosphate synthase (TPS1) gene of yeast expressed to lines of the potato cultivar White Lady showed improved drought tolerance. Transcriptome of wild-type and TPS1-transgenic plants were compared by using the POCI microarray containing 42,034 potato unigene probes to understand the molecular basis of this phenomenon. One major way to improve drought tolerance in plant species is to transfer genes encoding metabolic enzymes or transcription factors that utilize their effects through different mechanisms of action (Cattivelli *et al* 2008). Genes of various sources involved in trehalose metabolism have been used in some plant species to enhance their drought tolerance. Trehalose, consisting of two glucose molecules, is a very plentiful sugar in nature. In bacteria, yeast and tolerant crops it accumulates under stress condition and plant cells can survive by protecting membranes and proteins (Jain & Roy 2009 and Gilbert *et al* 2000). In other plants trehalose is synthesized at a different level. Trehalose is synthesized in *Escherichia coli*, yeast and plants, in a two-step process. Initially, trehalose-6-phosphate (T6P) is synthesized from glucose-6-phosphate (G6P) and UDP-glucose (UDPG) by trehalose phosphate synthase (TPS) and then T6P is changed into trehalose by trehalose phosphatase (TPP). They expressed that water and protein amount in transgenic plants did not change compared to the wild-type. Chlorophyll amount in transgenic leaves was a little, but not significantly, higher than in the wild-type leaves. But, shoot mass and leaf area of the TPS1-transgenic lines were about 35 and 24% lower, respectively, than in the wild-type. The 99 differentially transformed genes that we have identified in the microarray experiments were exported into the Map Man software for functional annotation. Of these 99 genes, 53 were determined into different functional categories, while the bin of "not determined" genes contains 46 genes of which 36 encode unknown, hypothetical proteins. Their microarray results showed that a sucrose synthase gene (SUS3) and six other genes associated with photosynthesis and carbon metabolism are regulated in TPS1 transgenic leaves. In 2007, Tang and coworkers investigated to creation transgenic potato plant for increasing tolerance to multiple environmental stresses by overexpressing nucleoside diphosphate kinase 2 gene. Four lines for every transgenic potato (*Solanum tuberosum* cv. Atlantic) plant (SN1, SN19 and EN1, EN2) with high tolerance to methyl viologen (MV, 10 LM) were used in that research. Plants were reproduced under sterile conditions in Petri dishes containing MS medium (Murashige & Skoog 1962) supplemented with 100 mg/L kanamycin. Then plants were transferred in pots and grown in a growth chamber under 16-h photoperiod with light intensity (100 Lmol m^{-2}s^{-1}), 60% (w/v) relative humidity at 25˚C. Two vectors were used to express AtNDPK2 gene with SWPA2 promoter or enhanced CaMV 35S promoter. For high temperature stress, four-week-old potato plants growing at 25˚C growth chamber were transferred to 42˚C for 20 h in the growth chamber. These treated plants were transferred to normal conditions (25˚C, 100 Lmol m^{-2}s^{-1}) for recovery from the stress. The tolerance of transgenic plants to high temperature stress was evaluated as the photosynthesis activity (Fv/Fm) and the fresh weight of the plants after treatment. To evaluate the salt tolerance, seedlings (NT, EN, and SN plants) were reproduced on MS medium in vitro. Salt stress was accomplished by transferring shoots to test tube containing MS medium (solid) supplemented with 80 mol/cm^3 NaCl. Plants were cultured in a growth chamber under a 16-h photoperiod with light intensity (100 Lmol m^{-2}s^{-1}) at 25˚C. Tolerance was evaluated by measuring the root length and the root dry weight after 20 days of treatment. The root dry

weight was measured after drying the sample in the dry oven at 70˚C for 48 h. To evaluate the tolerance of transgenic plants to oxidative stress NT and transgenic plants (SN, EN) were estimated for visible damage 5 days after spraying with solutions containing 0, 150, 200 or 250 LM methyl viologen (MV). MV is a typical ROS generating redox active compound, which has been used as non-selective herbicide. All plants showed a significant symptom of leaf damage in correlation with MV concentration. However, SN and EN plants showed reduced symptoms of damage compared to NT plants. Especially, young leaves from SN plants were unaffected even under high MV concentration. They successfully created transgenic potato plants with transferring AtNDPK2 gene with SWPA2 promoter or CaMV 35S promoter. Transgenic potato plants, especially SN plants under SWPA2 promoter exhibited enhanced tolerance to environmental stress including MV-induced oxidative stress, high temperature and salt stress.

The hypothesis of the investigation is the possibility of increasing the salinity tolerance of two potatoes cultivars (Agria) by production of GMO potato. Research question to be asked: does the mtlD gene introduction to potato's callus cells increase the salinity tolerance of potato plant (Agria cultivar)? We planned to produce a GMO potato by method of leaf disk method (Horsch *et al* 1985) and then to study the features of this product. The main aim of our investigation was suggestion of ways for increasing the potato plant tolerance to salinity and water stress by use of mtlD gene.

Material and Methods

Tubers of potato (*Solanum tuberosum* L.) cv. Agria were achieved from Agricultural Research Center of Bam (Iran). Tubers of potato cv. Agria were sterilized by sodium hypochlorite (10%) solution for 10 minutes then they were washed by distilled water two times for 3 minutes. These tubers were planted in pots which filled with sterilized (autoclaved) soil in 121°C for 20 minutes. The terminal meristem (leafs buds) were harvested after 10 days and were inserted on culture media of MS (Murashige & Skoog 1962) supplemented with 3% sucrose, 8% agar and 2,4-D (3 mg/L) for callus induction. These samples were stored at 20-25°C under white fluorescent lamps with 16h photoperiod. Mannitol, 1-phosphate dehydrogenase gene (mtlD, E.C.1.1.1.17) was used to create a transgenic potato plant, resistant to salinity stress. This gene was isolated from pCabmtlD plasmid (*E. coli*) and then was cloned in PBI121 plasmid. CaMV 35S is the most commonly promoter which used in the creation of abiotic stress tolerant plants (Romero *et al* 1997). *Agrobacterium tumefaciens* (LBA4404) was used to create transgenic plant as a vector (Holsters *et al* 1978) which the PBI121 plasmid transfers to it and stored in 28°C temperature. The leaves buds of potato plant were cut into discs then cultured in dark for 3 days on co-cultivation medium (Murashige & Skoog) which supplemented with 2.5 mg/L IBA (indol benzyl amino purine), 0.1 mg/L NAA (naphthalene acetic acid), 30 g/L sucrose and 8 mg/L agar. Base on leaf disc method, which initially was used by Horsch and his colleagues in 1985, in this investigation, the leaf discs were incubated in the diluted *Agrobacterium culture* for 20 minutes (Horsch 1985). This culture should be co-cultivated in the dark condition for 2 days. At next step, the leaf discs were transferred to Murashige and Skoog medium containing 300mg/L cefotaxime and 150 mg/L kanamycin. The cultured was stored in the light condition for 10 days. After this stage, samples were transferred to MS medium containing 4 mg/L 2,4-D to callus induction. To regenerate shoot, callus was incubated in culture media supplement with 2.5 mg/L BA and 0.1 mg/L NAA. The regenerated shoots were transferred to culture MS supplemented with 1 mg/L IBA.

The properties of genetic modified potatoes which were created in the previous stage were evaluated. To evaluate the features of these transgenic potatoes two ways were applied that include: 1. Polymerase chain reaction and 2. Physiological assessment of transgenic plants.

1. PCR (polymerase chain reaction)

The process of PCR include: 1.1 CTAB method for DNA extraction: the protocol of this method is described as follows. Cetyltrimethylammonium bromide (CTAB) extraction is a method of extracting plant DNA that removes Polyphenolics from plant cell walls (Aljanabi & Martinez 1997). Polyphenolics are compounds with long chains that resemble DNA and precipitate in similar ways. This process uses Chloroform:Isoamyl Alcohol, which is both volatile and toxic. In the process, isopropanol is also used. The CTAB method also uses liquid nitrogen as part of the extraction process (Wulff *et al* 2002). The important materials used in CTAB method include: CTAB buffer, Microfuge tubes, Mortar and Pestle, Liquid Nitrogen, Microfuge, Absolute Ethanol (ice cold), 70 % Ethanol (ice cold), Ammonium Acetate (7.5 M), 55˚C water bath, Chloroform:Isoamyl Alcohol (24:1).

1.2.Selection of primers; in this research the forward primer was 5′-GTCTAGATGAAAGCATTACATTTTGGCG-3′.

The reverse primer was

5′-CCGAGCTCCACCATT ATTGCATTGC-3′.

1.3.PCR cycle, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min using PCR Thermal Cycle.

1.4.PCR products were run on a 1% Agarose gel followed by staining with Ethidium bromide.

2. Physiological assessment of transgenic potatoes

The transgenic and non-transgenic potatoes were subjected to different NaCl treatments (0, 50, 100, 150, 200 mol/cm^3) each with three replications for 30 days.

The growth indicators include fresh weight, dry weight, height, numbers of tubers, total weights of tubers; harvest index, shoot weight and root weight were measured at the end of the stress period. The obtained data were analyzed by SPSS software.

Table 1. The mannitol concentration in leaves, stems and roots of non-transgenic and transgenic potatoes cv. Agria

	Mannitol concentration (µ mol.g^{-1} fresh weight)			
Plant lines	Leaf$_A$*	Leaf$_B$*	Stem	Root
+mtlD	1.32 ± 0.114	0.98 ± 0.115	0.5 ± 0.3	1.91 ± 0.463
-mtlD	0.03 ± 0.001	0.02 ± 0.0004	0	0.04 ± 0.002

*Leaf$_A$: leaves on the top of plant. Leaf$_B$: leaves on the lower parts of plant

Table 2. The effect of salinity stress on transgenic and non-transgenic potatoes cv. Agria growth

NaCl *	Plant**	Fresh weight (g)	Dry weight (g)	Height (cm)	Numbers of tubers	Total weights of tubers (g)	Harvest Index	Shoot weight (g)	Root weight (g)
0	-mtlD	87.1 ± 0.4	18.40 ± 2.1	48.1±0.45	6	56±1.33	64±1.11	25±0.34	6.1±0.7
	+mtlD	83.3 ± 0.8	17.78 ± 0.8	45.4±1.22	6	48±1.23	58±1.4	29.4±0.5	5.9±0.3
50	-mtlD	52.6 ±1.2	12.43 ± 1.2	20.6±1.12	4	33±0.68	63±1.28	16±0.7	3.6±1.2
	+mtlD	59.8 ± 0.6	15.23 ± 0.9	39.6±1.37	3	35±1.27	62±2.34	19.9±1.21	4.9±0.8
100	-mtlD	34.1 ± 0.5	8.23 ± 1.2	16.8±1.36	2	17±0.86	56±2.43	13.3±0.92	4.1±0.6
	+mtlD	46.2 ± 1.12	12.47 ± 0.8	25.7±1.18	4	26±1.85	63±2.11	16.6±0.89	3.6±0.5
150	-mtlD	23.2 ± 0.7	4.85 ± 0.6	10.2±0.43	1	10±0.26	76±1.76	11±0.81	2.2±0.2
	+mtlD	36.6 ± 0.92	11.63 ± 1.3	18.8±1.42	2	14±0.56	79±1.48	21.3±1.4	1.3±0.3

*mol.cm^{-3} ; **Transgenic plant (+mtlD) and non-transgenic plant (-mtlD)

Results

The results of this investigation were showed in Table 2. The mean comparison showed that there are significant differences between four salinity stress treatments (0, 50, 150, 200 mol/cm^3 NaCl) and also there were significant differences between two mtlD treatments (+mtlD, -mtlD) in all growth indicators, by recorded probability alpha = 0.010. Data analysis of all growth parameters indicated the negative effect of salinity stress (different NaCl concentration) on two potato lines (+mtlD and -mtlD). Noticeably, the recorded growth indicators reduction in +mtlD (GMO potato) line was recorded more than –mtlD lines. In –mtlD plants, salt stress reduced fresh weight by 39% in 50 mol/cm^3 NaCl, 61% in 100 mol/cm^3 NaCl, 73% in 150 mol/cm^3 NaCl, while in +mtlD potatoes salt stress decreased fresh weight by 28% in 50 mol/cm^3 NaCl, 44% in 100 mol/cm^3 NaCl, 56% in 150 mol/cm^3 NaCl. In –mtlD plants, salt stress reduced dry weight by 32% in 50 mol/cm^3 NaCl, 55% in 100 mol/cm^3 NaCl, 73% in 150 mol/cm^3 NaCl but in +mtlD potatoes salt stress decreased dry weight by 14% in 50 mol/cm^3 NaCl, 30% in 100 mol/cm^3 NaCl, 35% in 150 mol/cm^3 NaCl (see Table 2). Growth analysis is used logical tool for evaluating plant growth and in standard growth analysis, CGR was applied to determine the plant growth. CGR is defined as dry matter accumulation rate per unit land area. It has been calculated as follows: CGR = (W2- W1)/SA (t2 – t1) where CGR is crop growth rate showed in g per m^2 per day, W1 and W2 are crop dry weights at the beginning and end of intervals, t1 and t2 are corresponding days, and SA is the land area occupied by plants at each sampling. Values of CGR are normally low during early growth stages and increase with time, reaching highest values at the time of flowering. The CGR (Crop Growth Rate) for +mtlD is greater than –mtlD potatoes. The highest CGR for +mtlD was 12 g per m^2 and 8.5 g per m^2 for –mtlD (Fig. 1). Relative growth rate (RGR) is an evaluation used in plant physiology to measure the speed of plant growth. It is evaluated as the mass increase per aboveground biomass per day, for example as g g^{-1} d^{-1}. RGR is calculated using the following equation: RGR = (ln W2 - ln W1)/(T2-T1) where W1 and W2 are plant dry weights at times T1 and T2. Many plants show a decreasing RGR over time but the RGR for –mtlD is fewer than transgenic potato (Fig. 2). When comparing RGR values, it was determined that transgenic potatoes lines have a higher RGR than non- transgenic potatoes lines. The regression equation for salinity stress (NaCl concentration) with dry weight of transgenic (+mtlD) and non-transgenic potatoes lines (-mtlD) were created by SPSS software. The linear regression equations exhibit that the transgenic (+mtlD) has a low negative gradient (slope) in compare with non-transgenic (-mtlD). This declared the low negative influence of salinity on +mtlD potato due to creation of mannitol and osmotic pressure was increased in transgenic potato cv. Agria. Then +mtlD has more tolerance to salinity stress (equation 1 and equation 2).

Discussion

GMO potato (+mtlD), Agria cultivar, showed the more tolerance to salinity stress compared with non-transgenic potato (control treatment, -mtlD). It was observed that the osmotic pressure in transgenic potato cv. Agria was increased by production of mannitol which this approved the successfully expression of mtlD gene (mannitol-1-phosphate dehydrogenase, EC 1.1.1.17) to transgenic potato cv. Agria plant. The tolerance to salinity stress increased with increase of mannitol content and mannitol was significantly produced by existence of mtlD gene in transgenic potato cv. Agria in this investigation (Table 1 and Table 2). Similar results had also been reported by Thomas *et al* (1995). Thomas and his colleagues described expression of mtlD gene for biosynthesis of mannitol in *Arabidopsis thaliana*. They showed that tolerance to salinity of seeds increased due to accumulation of mannitol (Thomas *et al* 1995). Abebe and his coworkers, in 2003, also declared the expression of mtlD gene to wheat plant for increasing tolerance to salinity stress by production of mannitol (Abebe *et al* 2003). Karakas *et al* (1997) conducted an experiment to determine whether mannitol provides salt and/or drought stress protection through osmotic adjustment. In their study, Tobacco plants (*Nicotiana tabacum L.*) were transformed with a mannitol-1-phosphate dehydrogenase gene resulting in mannitol accumulation. Salt stress reduced dry weight in wild-type plants by 44%, but did not reduce the dry weight of transgenic plants. In 2007, Tang and coworkers investigated the creation of transgenic potato plant for increasing tolerance to multiple environmental stresses by overexpressing nucleoside diphosphate kinase 2 gene. They successfully created transgenic potato plants with transferring AtNDPK2 gene with CaMV 35S promoter. Transgenic potato plants, especially SN plants under SWPA2 promoter exhibited enhanced tolerance to environmental stress including MV-induced oxidative stress, high temperature and salt stress.

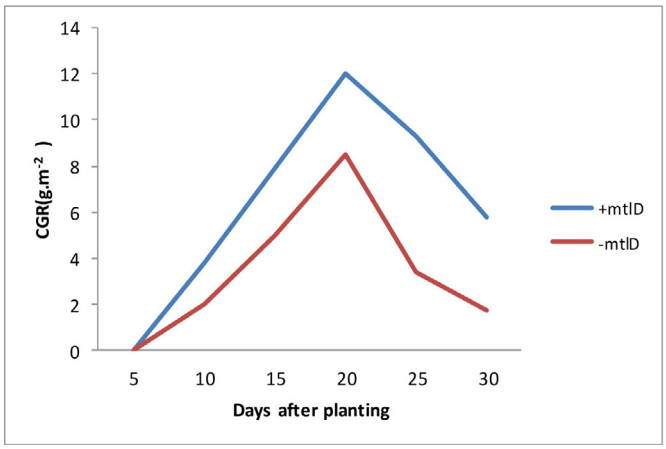

Fig. 1. CGR (Crop Growth Rate) of +mtlD and –mtlD potatoes cv.Agria (irrigated with 150 mol/cm^3 NaCl)

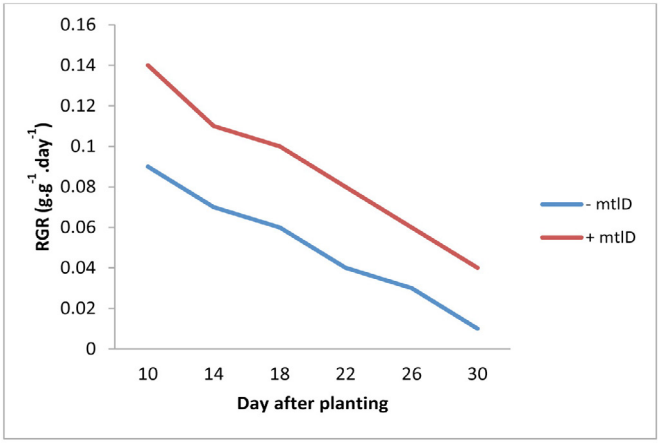

Fig. 2. RGR (Relative Growth Rate) of +mtlD and –mtlD potatoes cv.Agria (irrigated with 150 mol/cm^3 NaCl)

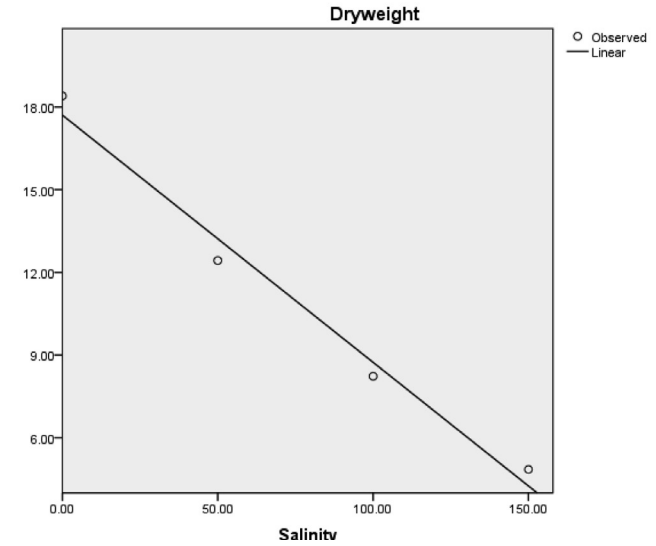

Fig. 3. Linear regression for salinity and dry weight of non-transgenic potato (-mtlD).

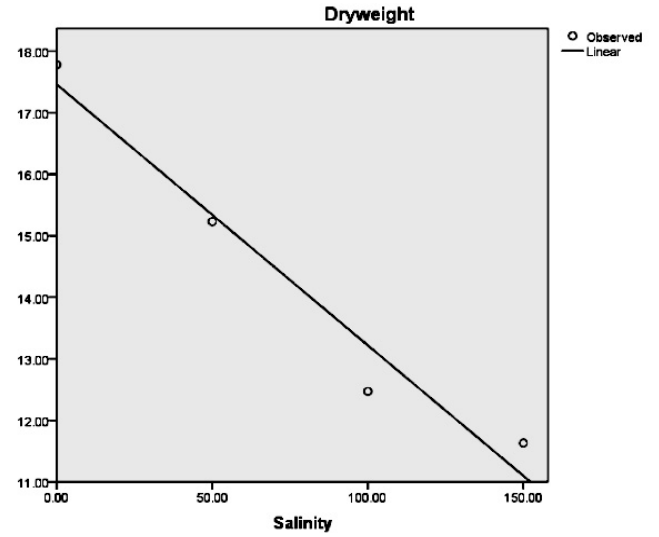

Fig. 4. Linear regression for salinity and dry weight of transgenic potato (+mtlD)

Moghaieb-Reda and coworkers, in 2011, from Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt, declared that they evaluated the salt tolerance in ectoine-transgenic tomato plants (*Lycopersicum esculentum*) in terms of photosynthesis, osmotic adjustment and carbon partitioning. In their study, the hypocotyl explants isolated from two tomato cultivars were transformed with the *Agrobacterium tumefaciens* LBA-4404 harboring the three genes involved in ecotin synthesis. This was stimulated in the leaves and roots by salt application, which improved water status by maintaining higher activities of water uptake and transport to leaves. Furthermore their results support hypothesis that ecotin alleviates inhibition of root sink activity at a first response to salinity.

Growth analyses include, CGR (Crop Growth Rate) and RGR (Relative Growth Rate) of transgenic and non-transgenic potatoes lines were showed that +mtlD potatoes cv. Agria lines grew better than –mtlD potatoes cv. Agria lines in salinity stress. This approved that +mtlD had more tolerance to salinity stress due to production of mannitol.

The comparison of linear regression equations of transgenic (+mtlD) and non- transgenic potatoes cv. Agria (-mtlD) showed the more tolerance of +mtlD to salinity stress than –mtlD potatoes cv. Agria (Table 3, Table 4, Figs 3-4).

On the whole, we can conclude that it was created GMO potato cv. Agria in this study. Also it was approved that mtlD gene transfer to GMO potatoes plants by Polymerase Chain Reaction and physiological parameters assessment. The GMO potatoes cv. Agria exhibited tolerance to salinity stress (even in 150 mol/cm^3 NaCl). This showed the expression of mtlD gene to genetic modified potatoes plant which causes producing mannitol and increase osmotic pressure (Table 2) and so tolerances to salinity stress of GMO potatoes were increased and this is in agreement with Karakas *et al* (1997), Huizhong *et al* (2000), Hu *et al* (2005), Khare *et al* (2010)and Moghaieb-Reda *et al* (2011). Transgenic plants exhibited mannitol concentrations up to 0.5–2 µmol/g of fresh weight, whereas mannitol accumulation could not be seen in untransformed potato (Table 1).

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Table 3. The equation of -mtlD potato (non-transgenic potato)
Equation 1. Y = 17.702 – 0.090 X

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Linear	0.983	114.896	1	2	0.009	17.702	-0.09
Logarithmic[a]	-	-	-	-	-	0	0
Power[a]	-	-	-	-	-	0	0

The independent variable is salinity and dependent variable is dry weight

Table 4. The equation of +mtlD potato (transgenic potato)
Equation 2. Y = 17.459 – 0.042 X

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Linear	0.959	46.965	1	2	0.021	17.459	-0.042
Logarithmic[a]	-	-	-	-	-	0	0
Power[a]	-	-	-	-	-	0	0

The independent variable is salinity and dependent variable is dry weight

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