



STRESS SIGNALS DETERMINATION IN DIFFERENT SWEET POTATO CULTIVARS AT SEEDLING STAGE UNDER SALT STRESS CONDITIONS

Mohamed H. Arisha^{1,2*} and L. Qiang¹

1. Xuzhou Inst. Agric. Sci., Jiangsu Xuhuai District, Sweet potato Res. Inst., CAAS/Key Laboratory of Biol. and Genet. Improv. Sweet Potato, Minist. Agric., Xuzhou 221131, Jiangsu, China

2. Hort. Dept., Fac. Agric., Zagazig Univ., Sharkia 44511, Egypt

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ABSTRACT: The first action taken by plant to cope with salt stress is producing fast action substances, *i.e.*, proline, malondialdehyde, peroxidase activity, *etc.*, which produced during the first phase of stress as a temporary defence response against stress. Sensitive and tolerant sweet potato cultivars are different in response to salt stress. Four sweet potato cultivars (Xu-28, Xu-Zi-3, Xu-22 and Yan-Zi-3) were used in this study to identify the differences in response to salt stress between tolerant and sensitive cultivars. Seedlings were treated with 200 mM of NaCl and samples were collected at 0, 30 min., 1, 3, 6, 12, 24, 48 and 72-hours after treatment. A modified standard evaluation score (SES) of visual salt injury was used to measure the degree of salt tolerance after 14 days of salt stress treatment. SES results indicated that Yan-Zi-3 and Xu-22 were salt sensitive cultivars, while Xu-28 and Xu-Zi-3 were tolerant cultivars. During the first phase of salt stress exposure, the proline accumulation in leaves of the two tolerant cultivars (Xu-Zi-3 and Xu-28) was higher than in sensitive cultivars (Yan-Zi-3 and Xu-22). Malondialdehyde level started to increase at 30 minutes after salts stress treatment and it was higher in Xu-22 and Yan-Zi-3 (9.253 and 9.590 nmol/ml, respectively) than those recorded in Xu-28 and Xu-Zi-3 (4.368 and 4.466 nmol/ml, respectively). In addition, protein synthesis was more pronounced in tolerant than sensitive cultivars. Peroxidase responding to salt stress in sweet potato appeared at only one time point differed according to cultivars. Furthermore, the increment level in both of tolerant cultivars was higher than that of sensitive cultivars.

Key words: Sweet potato, salt stress, physiological, biochemical parameters.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.), is mainly growing in more than 100 developing countries (Chandrasekara and Kumar, 2016). It is a low input crop that has a wide ecological adaptation. Furthermore, it is considered a rich source for vitamin-A and starch. It produces more biomass and nutrients per hectare than any other food crops in the world. Although it is underutilized crop till now, it is supposed to solve the problem of food deficit and malnutrition, especially in developing countries (Motsa *et al.*, 2015).

Therefore, it is considered as an important crop for food security worldwide.

Salt stress, a kind of abiotic stress, is widely playing a major role in determining the distribution of sweet potato. Furthermore, salt stress is considered as a main factors for sweet potato growth and productivity, especially in the arid and semi-arid regions (Ahanger *et al.*, 2017). It is caused due to the excess of Na⁺ and Cl⁻ that negatively affect plant growth and development through low osmotic potential of soil solution, nutritional imbalance, specific ion

*Corresponding author: Tel. : +201007857984
E-mail address: mohamedarisha@gmail.com

effect and a combination of these factors (Tavakkoli *et al.*, 2010). Mainly plant response to salt stress consists of numerous physiological and bio-chemical processes. During the first phase of salt stress, plants accumulate fast action substances or low molecular mass compounds and enzymes known as a compatible solute, proline, peroxidase (POD), malondialdehyde (MDA), mannitol, sorbitol glycine betaine. The degree of salt stress resistance in sweet potato plants depends on the amount of accumulation of these low molecular mass compounds, as well as enzyme activity (Rasool *et al.*, 2013).

Proline, a fast action substance produced in plants under salt stress conditions. It is considered as a primary defender against stress. It contributes to stabilize subcellular structure and buffer cellular redox potential under stress conditions. In sweet potato, as well as other plant species, proline accumulation has been correlated with stress tolerance and its concentration has been showed to be generally higher in salt tolerant cultivars than in salt sensitive cultivars (Zhang *et al.*, 2017).

Malondialdehyde (MDA) is one of the end-product of lipid peroxidation and salinity enhanced accretion. It increases in the case of environmental stress and is regarded as a marker for evaluation of plasma and organelle membranes damage (de Azevedo Neto *et al.*, 2006).

Proteins produced in plants with different ways under salt stress. They play an important roles in accelerating the cellular metabolism and maintaining homeostasis in plants under stress conditions (Yu *et al.*, 2018).

Peroxidase (POD) is one of the antioxidant enzymes that prevent the oxidative stress in plants under salt stress. It also plays an important role in avoiding cellular damage by creating active oxygen species in chloroplast (Ara *et al.*, 2013).

Furthermore, identifying the differences in sweet potato physiological responses between salt sensitive and tolerant cultivars which primarily depends on the genotype, considered an important aspect (Ashraf and Harris, 2004). The previous studies investigated the problems of plant response to salinity in many different crops. However, the response of sweet potato to

salinity was not well addresses. Hence, this paper presents a primary study to identify the differences between the response of tolerant and sensitive sweet potato cultivars under salt stress conditions.

MATERIALS AND METHODS

Plant Materials

Four sweet potato cultivars (Yan-Zi-3, Xu-22, Xu-28 and Xu-Zi-3), differ in their degree of salt sensitivity were used in this experiment. Yan-Zi-3, is a previously known as a salt sensitive cultivar, Xu-28 is known as a tolerant cultivar (Park *et al.*, 2017), while the degree of salt sensitivity in the other cultivars are not known. The sources of the four cultivars are sweet potato Rresearch Institute, Chinese Academy of Agriculture Science, China.

Treatments and Experimental Design

Apical stem cuttings (15-20 cm) of four sweet potato genotypes were grown in hydroponics culture using Hoagland nutrient medium for four weeks. Seedlings were exposed to salt stress (200 mM NaCl were added to Hoagland solution). 0.1 g sample were taken from the upper third leaf after 0.30 min., 1, 3, 6, 12, 24, 48, 72-hours and immediately frozen in liquid nitrogen for stress signals determination.

Visual Salt Stress Injury Evaluation

Scoring of visual salt stress injury of sweet potato seedlings treated with 200 mM NaCl was performed using a modified standard evaluation system from rice standard evaluation score (SES, Table 1) (Gregorio *et al.*, 1997).

Stress Signals and Enzyme Activity

Proline content

Samples were ground in liquid nitrogen to fine powder for the determination of proline content. Proline was extracted and the content was assayed spectrophotometrically using Indene tri-ketone colorimetric method (Bates *et al.*, 1973). Absorption of chromophore was read at wave length 520 nm using a 3200 UV/VIS spectrophotometer. The proline concentration was calculated using L-proline corresponding on standard curve.

Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but few leaves wilted and turned to bright or yellow colour	Tolerant
5	Growth severely retarded and most leaves wilted	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or drying	Highly sensitive

Malondialdehyde (MDA)

The content of Malondialdehyde (MDA) was determined by Thiobarbituric method (TBA) according to the kit manual (A003-3) from Nanjing Jiancheng Bioengineering Institute (**Draper et al., 1993**).

Peroxidase (POD)

Peroxidase activity was measured on the basis of determination of guaiacol oxidation (**Aebi, 1974; Lagrimini, 1991**). Enzyme specific activity was expressed as OD₄₂₀ nm.

Total protein

The protein was quantified by a colorimetric assay for protein assay kit (Jiancheng Bioengineering Institute, A-084-3). The absorption values were read at wave length of 562 nm with a 3200 UV/VIS spectrophotometer (**Böddi et al., 1996**).

Correlation Study

Pearson correlation was conducted for the average of the four cultivars during the different times points using Statistix 8.1 software (**Statistix, 2008**).

Statistical Analysis

The experiment was arranged in a randomized complete blocks design with three biological replicates. Statistical analysis was conducted for all collected data using SPSS 16.0 statistical software package (SPSS, Chicago, USA) according to Snedecor and Cochran (**Snedecor and Cochran, 1980**) and the least significant difference test ($P \leq 0.05$) was used to investigate the significant differences among the treatments for all tested parameters.

RESULTS

Salinity Tolerance Characteristics

The salinity tolerance score was calculated by using visual salt injury (Table 1) for four sweet potato cultivars (Xu-22, Xu-Zi-3, Xu-28 and Yan-Zi-3) grown in Hoagland solution with 200 Mm of NaCl for 14 days. The results in Table 2 and Fig. 1 indicate that Yan-Zi-3 was the most sensitive cultivar among the four evaluated cultivars with severe retarding growth, most of leaves were dry and some cuttings were died after 14 days of salt stress exposure (Salinity score = 8.125). Followed by Xu-22 which was significantly affected with symptoms of dry leaves and slow growth, but fewer plantlets were died (Salinity score = 7.825). Furthermore, Xu-Zi-3 and Xu-28 cultivars were not affected seriously by salt stress, and plantlets were growing near normally with few symptoms of yellowing and leaves wilting (Salinity score is 3.375 and 3.25, respectively).

In both sensitive cultivars (Yan-Zi-3 and Xu-22), the growth was strongly inhibited in the first phase of salt stress. Leaf wilting symptoms appeared clearly after 24 hours and gradually increased, while yellowing symptoms appeared after 48 hours of salt stress. Salt visual symptoms became clear at 7 days after salt stress treatment then after 14 days of salt stress treatment leaves became dry. In addition, shoot tips turned into yellow colour without new-leaf initiation after 14 days of salt stress exposure. Furthermore, in the tolerant cultivars (Xu-28 and Xu-Zi-3), wilting symptoms slightly appeared after 7 days of salt stress, followed by turning elder leaves into bright colour. At 14 days after

Table 2. Standard evaluation score (SES) in sweet potato plants after 14 days with 200 mM of NaCl treatment

Cultivar	Salinity score (Mean \pm SE)	Tolerance scale	Leaf colour
Xu-22	7.83 \pm 0.19 a	Sensitive	green
Xu-Zi-3	3.38 \pm 0.15 b	Tolerant	Purple
Xu-28	3.25 \pm 0.24 b	Tolerant	purple
Yan-Zi-3	8.13 \pm 0.53 a	Sensitive	green

SES Score: 1, tolerant; 9, highly susceptible; different small superscript letters indicate significant differences, determined by least significant difference (LSD) at P = 0.05.



Fig. 1. Visual salt injury on four sweet potato cultivars (Xu-28, Yan-Zi-3, Xu-22 and Xu-Zi-3) under salt stress (200 mM of NaCl); A, salt treated plants after 12 hours of salt stress as compared to control, B, salt treated plants after 7 days of salt stress as compared to control, C, control plants of the four sweet potato cultivars, D, salt treated plants after 14 days of salt stress

salt stress, the tolerant cultivars showed less injury symptoms as compared to the sensitive cultivars. In addition, the shoot tips were growing near to normal and new leaves were coming out without any severe symptoms (Fig. 1).

Stress Signals Assay

Fig. 2A show that salt stress induces a marked increase in proline accumulation as compared to control plants. Proline content started to increase with the same levels in both sensitive and tolerant cultivars after 30 minutes of exposure to salt stress (Ashraf and Harris, 2004). In both tolerant cultivars (Xu-Zi-3 and Xu-28), the proline content highly increased starting from one hour of salt stress, reaching to the peak at 6 hours with values of 96.48 and 66.15 $\mu\text{g/g}$ fresh weight, respectively, then after the proline content declined until 72 hours. Furthermore, in Yan-Zi-3 (a sensitive cultivar), proline content reached to the peak in leaves after 48 hours of salts stress (76.57 $\mu\text{g/g}$ fresh weight). While, in the other sensitive cultivar (Xu-22), the proline level increased with the same range as the other cultivars at 30 minutes. However, in Xu-22 the proline level continued slightly increasing until 72 hours of exposure to salt stress on sweet potato stem cuttings.

Therefore, the results indicated that MDA content in leaves increased after 30 min of exposure to salt stress. Furthermore, the increment level at 30 minutes was higher in Xu-22 and Yan-Zi-3 (9.253 and 9.590 nmol/ml, respectively) than that in Xu-28 and Xu-Zi-3 (4.368 and 4.466 nmol/ml, respectively). In addition, Xu-28 recorded the lowest MDA content among the four cultivars in all time points, followed by Xu-Zi-3. While, Xu-22 and Yan-Zi-3 recorded higher values of MDA content in all time points than other cultivars (Fig. 2B).

Fig. 2C show that the protein level increased in salt stressed sweet potato plants as compared to control plants in all time points. The effect of salt stress on protein synthesis was more pronounced in tolerant cultivars than sensitive cultivars. Furthermore, after 30 minutes of salt stress exposure, the protein synthesis increased giving the highest values in the two tolerant cultivars (Xu-Zi-3 and Xu-28, valued 2879.83 $\mu\text{g/ml}$ and 28866.1818 $\mu\text{g/ml}$, respectively),

followed by the sensitive cultivar of Yan-Zi-3 with value of 2197.41 $\mu\text{g/ml}$. While, in Xu-22 the protein content started to increase after 1 hour of salt stress giving 1346.65 $\mu\text{g/ml}$. In addition, the protein level reached to the peak in the tolerant cultivars; Xu-Zi-3 and Xu-28 with values of 3782.90 and 4533.57 at 3 and 24 hr., after salt stress, respectively. In the sensitive cultivars, Yan-Zi-3 and Xu-22 protein content reached to the peak with values of 3446.24 and 3619.12 at 12 and 48 hr., respectively.

Fig. 2 D show that POD content increased reaching to the peak at 30 minutes, 1, 6 and 24-hours of salt stress in the four cultivars with values of 1056.55, 1121.51, 999.16 and 1150.07 U/mg protein, respectively. While, POD content was at the normal range at the other time points (Fig. 2 D).

Simple Person's Correlation

Correlation coefficients among salt stress signals and enzyme activity in the four sweet potato cultivars were analysed by Person correlation (Table 3). Proline positively correlated with MDA content ($r=0.8$ at $p<0.01$). Furthermore, the total protein contents positively correlated with nitrogen contents ($r=1.0$ at $p<0.01$) in Xuzishu-8 under salt stress conditions. MDA content displayed a positive correlation with total protein and nitrogen ($r=0.508$ and $r=0.508$, respectively) at $p<0.05$ at the time of exposure to salt stress. Contrarily, POD was negatively correlated with total protein and nitrogen ($r=-0.52$ and -0.52 , respectively) at $p<0.05$.

DISCUSSION

In this study, SES index of visual salt injury was employed to characterise salt tolerance in sweet potato such as rice (Chunthaburee *et al.*, 2016). Yan-Zi-3 is a previously identified sensitive cultivar, therefore it is used as a sensitive control and Xu-28 previously investigated as a tolerant cultivar, so it was used as a tolerant control (Park *et al.*, 2017). SES index indicated that the cultivars (Yan-Zi-3 and Xu-22) are sensitive and the other two cultivars are tolerant (Xu-Zi-3 and Xu-28). In addition, the visual evaluation results indicated that there are differences in the response to salt stress between salt tolerant and sensitive sweet potato cultivars

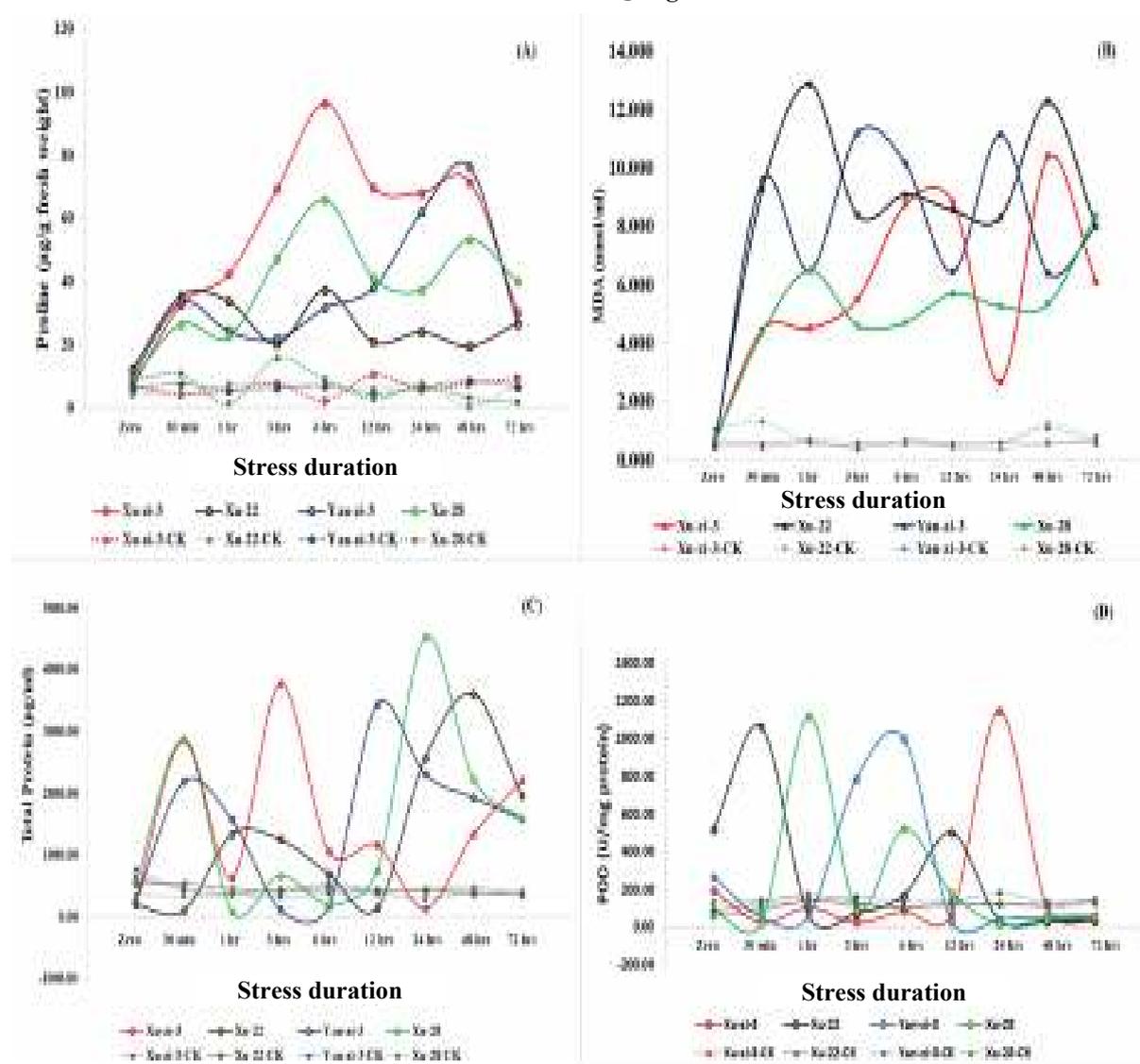


Fig. 2. Effect of salt stress on stress signals and enzymes activity; A, Proline content, B, Malondialdehyde (MDA), C, Total protein, D, Peroxidase activity (POD).

Table 3. Pearson's correlation coefficient among physiological parameters from four sweet potato cultivars exposed to 200 mM of NaCl. Each square indicates the person's correlation coefficient of a pair of parameters

	Proline	MDA	POD	Total Protein	Nitrogen
Proline					
MDA	0.80**				
POD	0.07	-0.13			
Total Protein	0.40	0.51*	-0.52*		
Nitrogen	0.40	0.51*	-0.52*	1**	

In salt sensitive cultivars, the growth was strongly inhibited, wilting and yellowing symptoms seriously appeared and at 14 days of salt stress exposure most of the leaves dried or seedlings almost died. However, injury symptoms as affected by salt stress appeared later in the tolerant cultivars and shoot tip leaf initiation and extension wasn't significantly affected. These results are in agree with those of **Farida et al. (2015)** who proved that the response of different sweet potato genotypes differ in the response to salt stress. In addition, they also found that these differences may be due to the K^+/Na^+ level. Similar results were observed in maize (**Murat et al., 2010**), wheat (**Begum et al., 2008**), and rice (**Chunthaburee et al., 2016**).

The results showed that during the first phase of salt stress exposure, the proline accumulation in leaves of the tolerant cultivars (Xu-Zi-3 and Xu-28) was higher than that of sensitive cultivars (Yan-Zi-3 and Xu-22). Highly proline accumulation in tolerant cultivars contributed to reduce or delay the harmful effect of salt stress (**Lichtenthaler, 1998**). Similar results were found by **Gharsallah (2016)** who clarified that during the first phase of salt stress, plant produce proline as a fast action substance in order to cope with stress. In addition, **Zhang et al. (2017)** found that plant tolerance improved through increased proline and abscisic acid contents in leaf tissues during the first phase of salt stress in sweet potato. However, **Chunthaburee et al. (2016)** illustrated that proline accumulation didn't improve salt tolerance in rice after 14 days of salt stress. This is occurred because the proline role is reducing stress effect during the first phase of stress only (**Maggio et al., 2002**). Later, when the plant identify the stress type, it uses other different mechanisms to cope with stress (**Ashraf and Harris, 2004; Munns and Tester 2008**). Furthermore, previous studies were proved that there was a relation between sensitivity or tolerance and proline accumulation in leaves during the first phase of stress (**Delauney and Verma, 1993; Verbruggen and Hermans, 2008**).

Malondialdehyde (MDA), is one of the end-products of lipid peroxidation in bio-membranes. MDA is usually used to represent the level of lipid peroxidation and membrane injury (**Draper**

and Hadley, 1990; de Azevedo Neto et al., 2006). In the current results, MDA content in the leaves of the sensitive cultivars (Yan-Zi-3 and Xu-22) reached two folds higher than that in tolerant cultivars (Xu-Zi-3 and Xu-28). High MDA content in the sensitive cultivars reflect the statues of membrane damage due to lipid peroxidation. These results explains clearly the appearance of visual symptoms including yellowing, wilting and injuries on seedlings exposed to salt stress. The lower level of MDA concentration in Xu-Zi-3 and Xu-28 demonstrates that the plant may have better defend action against oxidative damage under salinity stress.

Protein content was increased in sweet potato leaves during salt stress, it plays an important role for accelerating the cellular metabolism and maintaining homeostasis (**Yu et al., 2018**). That's contribute in reducing the cell damage occurred by stress that enhanced salt tolerance (**Kosová et al., 2013**). In addition, the results pointed out that protein synthesis in salt-tolerant sweet potato cultivars was higher than in salt-sensitive cultivars under salt stress at all time points, which indicate that the metabolism rate was higher in tolerant than sensitive cultivars. In addition, higher protein synthesis in tolerant cultivars plays an important role in osmotic adjustment against salinity (**Sen and Alikamanoglu, 2011**).

POD plays an important role in preventing cellular damage by increasing re- active oxygen species in chloroplast (**Corpas et al., 2015**). In the current results, POD responding to salt stress in sweet potato appeared at only one time point among all time points in one cultivar. POD reached to the peak at 30 minutes, 1, 6 and 24-hour in Xu-22, Xu-28, Yan-Zi-3, and Xu-Zi-3, respectively. Furthermore, the increment level in both of tolerant cultivars (Xu-Zi-3 and Xu-28) was higher than sensitive cultivars (Xu-22 and Yan-Zi-3), which explain the ability of the tolerant cultivars in preventing cellular damage is higher than sensitive cultivars. These results are in agreement with the results of **Sai Kachout et al. (2009)** who found that adaptation of tolerant cultivars to salt stress increased through cell protection and higher peroxidase level. Furthermore, the antioxidant enzymes, such as POD are important components in preventing the oxidative stress (**Ara et al., 2013**).

The correlation studies of the current results showed that there was a negative correlation between MDA and POD, while there was a positive correlation between MDA and proline. These findings indicated that the salt stress tolerant enhanced by increasing POD. Increasing proline content indicates exposing the plant for higher salt stress. In addition, lower MDA content indicates lower damage percent of cellular components. In agree with these results **Chunthaburee et al. (2016)** showed that salinity stress generally induce damage to plant tissues. Also, the results indicated that POD play an important role in plant adaptation to stress, while MDA indicates more cellular damage.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Li Qiang, Zhang Yungang and Arisha designed the study. Arisha conducted the experiments, analyzed the results performed the figures and wrote the manuscript. Li Qiang, Liu Yaju and Wang Xin revised the manuscript. Yan Hui, Yan Qiang Qiang, and Gao Run Fei assisted in the experimental procedures. Kou Meng and Tang Wei assisted in the statistical analysis.

All authors read and approved the final manuscript.

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تحديد إشارات الإجهاد في أنواع مختلفة من البطاطا في مرحلة الشتلات تحت ظروف الإجهاد الملحي

محمد حامد عريشة^{٢٠١} - لي تشيانج^١

- ١- قسم تربية البطاطا - معهد أبحاث البطاطا، - الأكاديمية الصينية للعلوم - مدينة شوجو - مقاطعة جيانسو - الصين
- ٢- قسم البساتين - كلية الزراعة - جامعة الزقازيق - مصر

يتمثل رد الفعل الأولي الذي يتخذه النبات في مواجهة الإجهاد الملحي في إنتاج مواد سريعة الحركة، مثل البرولين، مالونديالديهيد، ونشاط البيروكسيداز، إلخ، والتي أنتجت خلال المرحلة الأولى من الإجهاد كرد فعل دفاعي مؤقت ضد الإجهاد، تختلف أصناف البطاطا الحساسة والمقاومة في استجابتها للإجهاد الملحي، تم استخدام أربعة أصناف من البطاطا الحلوة في هذه الدراسة (Xu-22 و Xu-Zi-3 و Yan-Zi-3 و Xu-28) لتحديد الاختلافات في الاستجابة للإجهاد الملحي بين الأصناف المقاومة والأصناف الحساسة، تمت معاملة الشتلات بـ ٢٠٠ ملي مول من كلوريد الصوديوم وتم جمع العينات في صفر و ٣٠ دقيقة و ١ و ٣ و ٦ و ١٢ و ٢٤ و ٤٨ و ٧٢ ساعة من المعاملة، تم استخدام مقياس ثابت معدل (SES) للتقييم البصري لمدي الإصابة و قياس درجة التحمل بعد ١٤ يوماً من معاملة الإجهاد الملحي. أشارت نتائج SES إلى أن Xu-22 و Yan-Zi-3 كانا من الأصناف الحساسة للملح، بينما كان Xu-28 و Xu-Zi-3 من الأصناف المتحملة للملح، أظهرت النتائج أن التعرض لإجهاد الملح خلال المرحلة الأولى أدى إلى تراكم البرولين في أوراق الأصناف المقاومة (Xu-28 و Xu-Zi-3) أعلى منه في الأصناف الحساسة (Xu-22 و Yan-Zi-3)، بدأ مستوى مالونديالديهيد في الزيادة في ٣٠ دقيقة بعد المعاملة وكان أعلى في Xu-22 و Yan-Zi-3 (٩,٢٥٣ و ٩,٥٩٠ مللي مول، على التوالي) من تلك الموجودة في Xu-28 و Xu-Zi-3 (٤,٣٦٨ و ٤,٤٦٦ مللي مول، على التوالي). بالإضافة إلى ذلك، كان تخليق البروتين أكثر وضوحاً في الأصناف المتحملة للملح أكثر من الأصناف الحساسة، ظهر نشاط البيروكسيداز في وقت واحد فقط نقطة تختلف وفقاً للأصناف كنتيجة للإجهاد الملحي في البطاطا، علاوة على ذلك، كان مستوى الزيادة في كلا الصنفين المقاومة أعلى منه في الأصناف الحساسة.

المحكمون:

- ١- أستاذ الخضر المتفرغ - كلية التكنولوجيا والتنمية - جامعة الزقازيق.
- أستاذ الخضر - كلية الزراعة - جامعة الزقازيق.

- ١- أ.د. عصام حسين أبو الصالحين
- ٢- أ.د. داليا أحمد سامي نوار