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Effects of Salinity on Soybean (*Glycine max* [L.] Merr.) DT26 Cultivar

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Abstract: Nowadays, climate change is the serious environment problem affecting the Earth. Higher earth temperatures melt iceberg raising the sea-level that causes salinity. Effects of salinity on growth and development of plants, specially crops, are one of the most concerns of plant physiologists. It has been proven that the difference among cultivars provides important sources for high quality breeding. Soybean (*Glycine max* [L.] Merr) is one of the few plants that can supply all eight essential amino acids. For a long time, it has become a very important crop in Vietnam. The effects of salt stresses ranging from lower to higher levels established by a gradient of NaCl concentration on the growth of soybean DT26 cultivar were studied. DT26 is the most popular cultivar of soybean used by farmers in Vietnam. The rate of germination, the length and fresh weight of shoots and roots, the content of chlorophyll and the content of proline were assessed in this study. Generally, at low salt concentration, the length of roots, the fresh weight of both shoots and roots increased, but under high salinity conditions these parameters were decreased. And, tissues of soybean DT26 cultivar accumulated more proline under saline condition.

Keywords: Soybean, *Glycine max*, DT26, salinity.

1. Introduction

Soybean seed has high protein and oil content and the unique chemical composition. Its protein has great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol. Soybean is one of the most valuable agronomic crops in the world. It is also used as a raw material for many human health care and industrial products. Soybean is classified as a moderately salt sensitive crop so its productivity is significantly hampered by salt stress [1-4].

Soybean DT26 cultivar was culled from various cross between DT12 and DT2000. It has yellow seeds, about 50-60cm plant height, branching to 2.0-2.5 stems/plant; average ratio of 18-22% three-seed fruits, growth duration average of 90-95 days. DT26 has become a national standard variety since 2008 with some advantages such as high content of protein (42.21%) and lipid (19.72%), less disease, and high yield. The yield is from 22-28 kg/ha to 30-32 kg/ha depending on farming condition.

In the field, the salinity of soil water or irrigation water is measured in terms of its

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electrical conductivity or osmotic potential [2]. Some kinds of plants are halophytes which are native to saline soils and complete their life cycles in that environment. The large majority of phant species ara glycophytes, which are not salt – tolerant and are fairly damaged by high salinity [2, 5]. In salinity, water and mineral nutrition uptake processes in plant are hampered because of change in osmotic potential relations between plant cells and surround environment. Salinity depresses growth and photosynthesis in sensitive species. The photosynthetic pigments of sensitive plants were reduced in salinity [6-8]. Furthermore, toxic ions of high saline conditions inhibit the activity of enzymes and growth stimulants [2, 4, 5].

In this study, a gradient of salinity ranging from 0mM, 50mM, 100mM to 200mM NaCl was used to assess the effects of salt stress on the growth and development of soybean DT26 cultivar. The rate of germination, the length and fresh weight of shoots and roots, the content of chlorophyll and the content of proline were assessed in this study. The comparison between plants in non- stress condition and plants treated with NaCl was carried out.

2. Materials and Methods

2.1. Plant materials and salinity treatment

Glycine max L. [Merr.] DT26 cultivar used in this study was provided by Legumes Research and Development Center, Field Crops Research Institute (FCRI). Soil purchased from Thuy Cam Company Limited was prepared in 21cm diameter and 15cm high pots. 10 seeds were sown per pot. Every pot was watered with 30mL Hoagland solution (developed by Hoagland in 1938 [9] and revised by Hoagland and Arnon in 1950 [10]) everyday. The concentration of NaCl in watering solution was prepared in a gradient including 0mM, 50mM, 100mM and 200mM. There were 5 pots for each treatment. Three replications were done for whole experiment. Germination rate was counted after 3, 5 and 7 days of treatment. After 7 days, the seedlings were taken to evaluate some preliminary physiological parameters such as the shoot length, the root length, the fresh weight of shoots and roots under salt stress.

2.2. Estimating leaf chlorophyll content

Chlorophyll (Chl) was extracted from leaf tissues using 80% aceton and measured the absorbances at 646nm and 664nm in spectrophotometer. Concentrations of Chl a and Chl b were calculated using the formula of Robert J. Porra, 2006 [11].

Chl a (µg/ml) = 12.7*A664 – 2.69*A646

Chl b (µg/ml) = 22.9*A646 – 4.68*A664

2.3. Proline measurement

Detached leaf, stem and root tissues were used as samples. 50mg of each sample was homogenized, and then 1 ml of sulfosalicylic acid (3%) was added, supernatant was collected by centrifugation. Mixture of supernatant, acid-ninhydrin (dissolve 0.1 g ninhydrin in 2.4 ml acetic acid; add 1.6 ml 6 M phosphoric acid) and acetic acid in the ratio of 1:1:1 was incubated at 100°C for 1 hour. Toluene was added to extract upper phase of reaction. The extraction of samples was measured in spectrophotometer at 520 nm against pure toluene [12]. Proline content in sample was calculated by comparison with a calibration curve prepared with different concentrations (0, 25, 50, 75, 100 µM) of standard L-proline (Merck).

3. Results and Discussion

3.1. Effects of salinity on germination of soybean DT26 cultivar seeds

Soybean seeds of DT26 cultivar were sown in different NaCl concentrations such as 0mM, 50mM, 100mM, and 200mM. The effects of different salinity conditions on soybean seeds

were clearly shown through the decrease of germination rate (Table 1). While under normal condition, the seed germination rate ranged from 93.7% to 97.2% after 3 to 7 days, the percentages of germinating seeds were reduced much more with increasing of NaCl concentration. Seed germination rates reduced 1.1 fold under 50mM NaCl at all time points and reduced from 1.6 to 1.7 fold under 100mM NaCl in comparison to control. High levels of salinity up to 200mM NaCl reduce the germination ability to 2.8; 2.5 and 2.3 times after 3, 5 and 7 days of treatment, respectively. Germination rate under stress can be sometimes considered as stress tolerance ability of cultivar [3-5].

Table 1. Effects of salinity on germination rate of soybean DT26 cultivar seedlings.
Data represents the means and standard errors of three independent experiments; 50 seeds were used each time. The comparison between salinity conditions and normal condition was determined by Student's t-test with significant difference (★) as p <0.05

Time	Concentration of NaCl in watering solution			
(days)	0 mM	50 mM	100 mM	200 mM
3	93.750 ±3,381*	84,259±4,243*	56.944±6,365*	33,333 ±8,333*
5	95.833 ±1,307*	85,185±2,778*	56.944 ±6,365*	38,889 ±4,811*
7	97.222 ±3,381*	86,111±5,782*	56,944 ±2,406*	41,667 ±8,333*

All of the reductions in germination rate of DT26 in high saline conditions were significant with p-value of student's t-test smaller than 0.05.

3.2. DT26 plant growth parameters were affected by salt stress

Salinity reduced the elongation of shoots in all concentrations of NaCl, but moderately salinity levels (50mM and 100mM NaCl) induced the length of roots, the fresh weight of both shoots and roots of DT26 soy plants (Figure 1). The shoot length under normal condition was about 40 cm, decreased to 34-35cm in 50mM and 100mM NaCl, respectively and to about 30cm in 200mM NaCl treatment (Figure 1A). On the other hand, concentrations of 50mM and 100mM NaCl made plant roots increase their length to 1.27 and 1.5 times in comparison to that of control. But at higher level of 200mM NaCl root length was only about 9cm, corresponding to 86% shorter than control's roots.

The fresh weights of both root and shoot tissues increased in 50mM and continuously increased in 100mM NaCl. But in 200mM NaCl condition the fresh weight was even less than that of in control condition. The highest fresh weight was achieved in 100mM NaCl treatment in shoots and also roots. And it was 1.363g of shoots and 0.271g of roots.

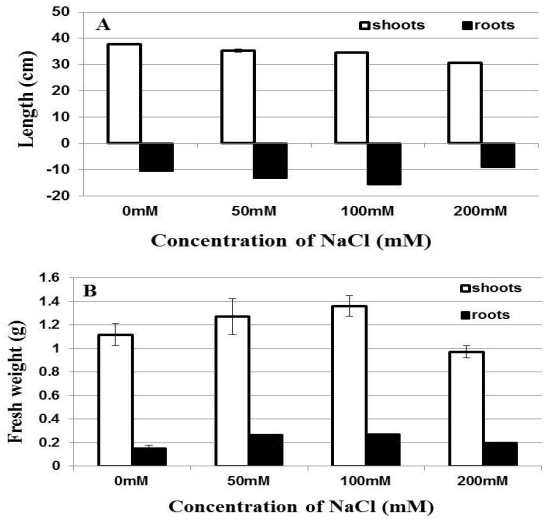

Figure 1. Effects of salinity on the length (A) and the fresh weight (B) of shoots (white bars) and roots (black bars) of soybean DT26 cultivar plants.

3.3. Content of chlorophyll a was more sensitive than chlorophyll b in salt stress

Leaf chlorophyll content provides valuable information about physiological status of

plants. So, the chlorophyll content was estimated in DT26 cultivar in this study (Figure 2.). Soybean reacts somehow like other species such as rice [6], rosy periwinkle [7] or poplar [8]… The increase of salt concentration in watering solution inhibited the chlorophyll accumulation. Control plants had high content of chlorophyll, 3.5 µg/mg of total Chl a and Chl b. Both Chl a and Chl b reduced their contents under salinity like reported previously [5-7]). And it was obviously that Chl a was more sensitive than Chl b to salt stress. When increasing salt concentration the Chl a content was reduced 89.4%, 79.0% and 67.1% in comparison with that of in normal growth plants. At the same time, Chl b content only reduced 91.9%, 79.9% and 73.6% in 50mM, 100mM and 200mM of NaCl concentration, respectively.

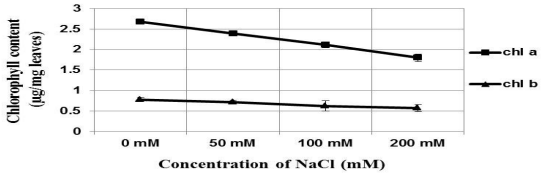

Figure 2. Effects of salinity on chlorophyll accumulation in soybean DT26 cultivar leaf tissues. Chlorophyll a _ line with squares and chlorophyll b _ line with triangles.

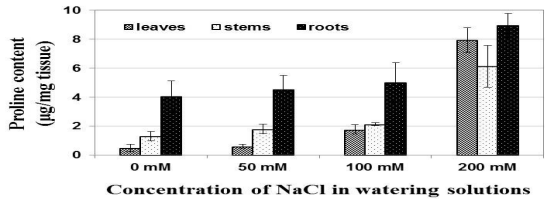

Figure 3. The proline contents in leaves (dash bars), stems (black dotted white bars) and roots (white dotted black bars) of soybean DT26 cultivar seedlings germinated after 5 days in different salinity conditions.

3.4. The content of proline of soybean DT26 under salt stress conditions

Using all kinds of tissues, the proline contents were evaluated and the result was presented in Figure 3.

The proline was mainly accumulated in roots of soybean DT26 cultivar in compare to leaves or stems. In normal condition) the proline content in roots was 3 fold higher than that in stems and more than 8 fold higher than that in leaves of seedlings. In salinity, in general seedlings had higher proline content (Figure 1.) as same as the previous reports [1-4]. For DT26, all seedlings of soybean induced more proline than in control in all type of tissues such as root, stem and leaf in salinity. The contents of proline in roots increased from 4.048 µg/mg to 4.528, 5.015 and 8.933 µg/mg with increasing of NaCl from 0mM to 50mM, 100mM and 200mM, respectively. While the content of proline in leaves increased only 1.24 times and 3.65 times in 50mM and 100mM NaCl, it was suddenly came up to more than 16.33 times in 200mM NaCl (7.922 µg/mg tissue) in comparison to that in non-stress condition (0mM NaCl). Proline content of stems was increased under salt stress, which was more than that observed in roots but less than that in leaves. Proline content in stems was 1.802 to 2.135 µg/mg tissue in 50mM to 100mM NaCl conditions, and was 6.124 µg/mg tissue in 200mM NaCl (about 4.7 times higher than in control). It seems that roots of soybean plant accumulate more proline than other kinds of tissues, but leaves are more affected by salinity than both stems and roots.

4. Concluding remarks

Salinity affected germination and other physiological parameters concluding growth, water uptake, chlorophyll content and also proline content in soybean DT26 cultivar. The reduction in the rate of germination, the length of shoots and the content of chlorophyll a and b in salinity was observed. At low salt concentration, an increase was noted in the length of roots, the fresh weight of both shoots and roots, but under high salinity conditions these parameters were decreased. All tissues of

soy plant accumulated more proline under saline condition. And leaves were more affected by salinity than both stems and roots.

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Ảnh hưởng của điều kiện mặn đến cây đậu tương (*Glycine max* [L.] Merr.) giống DT26

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Tóm tắt: Biến đổi khí hậu hiện đang là một vấn đề môi trường ảnh hưởng nghiêm trọng đến trái đất. Nhiệt độ tăng làm tan băng kéo theo nước biển dâng và gây ngập mặn.mặn gây ảnh hưởng tới sự sinh trưởng và phát triển của thực vật, đặc biệt là cây trồng, và đây cũng là mối quan tâm lớn của các nhà sinh lý thực vật học. Tuy nhiên, cây trồng rất đa dạng về chủng/giống. Và, sự khác biệt giữa các giống cũng rất hữu ích cho công tác chọn tạo giống chất lượng cao. Đậu tương (*Glycine max* [L.] Merr.) là một trong số ít loài thực vật có khả năng cung cấp đầy đủ các loại axit amin thiết yếu cho

con người. Từ lâu nay, đậu tương đã trở thành cây lương thực thực phẩm quan trọng của nước ta. Nghiên cứu này đánh giá ảnh hưởng của một số mức độ mặn được thiết lập nhờ xử lý tưới bằng dung dịch có bổ sung NaCl ở các nồng độ khác nhau cho giống đậu tương DT26. Đây là giống đậu tương được trồng phổ biến nhất ở Việt Nam. Các thông số về tỉ lệ nảy mầm, chiều cao chồi và rễ, hàm lượng chlorophyll trong lá và hàm lượng proline trong các loại mô rễ, thân, lá đều được đánh giá trong nghiên cứu này. Nhìn chung, ở mức mặn thấp, chiều dài rễ và trọng lượng tươi của cả thân và rễ đều tăng nhưng với độ mặn cao các chỉ tiêu này đều giảm. Các mô của đậu tương DT26 đều tích lũy nhiều proline hơn dưới tác động của độ mặn cao.

Từ khóa: Glycine max, giống DT26, điều kiện mặn.