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Evaluation of the Proline Content in Tissues of Soybean (*Glycine max* [L.] Merr.) DT26 Cultivar During Salt Acclimation

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Abstract: Salinity is one of the abiotic stresses that reduces the growth and development of plant. Soybean (*Glycine max* [L.] Merr.) is known to be sensitive to salinity; not only agronomy traits but also nodulation of soybean plant are inhibited in high salt concentration, thus reduce the yield of soybean. To cope with salt stress, soybean has developed several tolerance mechanisms. One of those is accumulation of comparative solutes which induce high osmotic potential for plant cells. Proline considered as a comparative solute was reported to play a critical role in increasing salt tolerance. However, knowledge about salt acclimation, the phenomenon of increase salt tolerance after exposing to salt stress at lower level before, are limited. Here, the changes of proline during salt acclimation in germination stage of soybean DT26 variety were studied. Proline content of salt acclimation and non-acclimation samples were compared to find out the role of acclimation in inducing salt tolerance in soybean through accumulation of proline. The results indicated the actually enhancement of proline biosynthesis during salt acclimation but it really differed from tissue to tissue of soybean plant.

Keywords: Proline, salt acclimation, salt tolerance, soybean, *Glycine max*, DT26.

1. Introduction

Salinity is an abiotic factor that limits plant growth and development [1-2] and it has become a serious agricultural problem. Salinity hampers plant not only by changing the relative water osmotic potential but also by breaking the ion balance between plant cells and surround environment [1-4] When exposing to salt stress, plants firstly loss the ability to absorb water then they are wilt because of osmotic stress as in drought [1, 3-6]. The second way of harmfulness is the high concentration of Na+ that causes severe ion toxicity [1-2]. In nature most of salinity is induced by high concentration of NaCl.

The accumulation of some compatible solutes in plant during salinity or drought could make the relative cellular osmotic potential to retain the water absorb ability [3, 5-8]. Proline is one of the compatible solutes like those. There were many reports mentioned about the accumulation of proline in salt tolerance of soybean [1, 4, 6, 9, 10]. Proline accumulated in both leaf and root tissues of tomato under salt stress [5]. The increase of proline content could induce the protection against the osmotic stress generated by salinity also in Arabidopsis, barley and poplar [6, 8, 11]. Germination stage is very

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sensitive period during plant life cycle and the salt tolerances induced at germination of many crops were researched [7, 12]. The difference of varieties of soybean plants had been analyzed under saline conditions before [12].

In this study, at the stage of germination, potted soybean (*Glycine max* L. [Merr.]) DT26 cultivar seedlings were watered with Hoagland solution supplemented with 0mM, 50mM, 100mM, 200mM NaCl. The seedlings germinating in high concentration of NaCl were considered as salt acclimated explants. The seedlings germinating in 0mM NaCl were non-acclimates. The comparison between proline contents under further salt stress (the second time) as well as the damage of plants during salt stress in growth stage were analyzed.

2. Materials and Methods

2.1. Plant materials

Seeds of *Glycine max* L. [Merr.] DT26 cultivar used in this study were provided by Legumes Research and Development Center, Field Crops Research Institute (FCRI). Seeds were potted in Thuy Cam soil (Thuy Cam Company Limited) – 10 seeds per pot with 21cm diameter and 15cm high. The seedlings were watering with 30mL Hoagland solution (developed by Hoagland in 1938 [13] and revised by Hoagland and Arnon in 1950 [14]) per pot every day.

2.2. Salt treatment and list of sample types

Seeds were germinated in saline solution which was made by supplement of different concentration of NaCl (0mM, 50mM, 100mM and 200mM). 7 days after sowing, seedlings, which had 3-5 real leaves, were continuously treated with equal or higher concentration of NaCl. So, there were 10 types of transferring explants as described in Table 1. There were 5 pots tested for each treatment. Three replications were done.

2.3. Rating the damages under salt stress

Plants were evaluated by looking at the symptoms of plants. Standard evaluation score (SES) of IRRI used to assess the visual symptoms of salt toxicity [15] was presented in Table 2.

Table 1. List of explant types tested in study.

Explant type	Salt condition during germination	Salt condition during development
A0-S0	0mM NaCl	0mM NaCl
A0-S50	0mM NaCl	50mM NaCl
A0-S100	0mM NaCl	100mM NaCl
A0-S200	0mM NaCl	200mM NaCl
A50-S50	50mM NaCl	50mM NaCl
A50-S100	50mM NaCl	100mM NaCl
A50-S200	50mM NaCl	200mM NaCl
A100-S100	100mM NaCl	100mM NaCl
A100-S200	100mM NaCl	200mM NaCl
A200-S200	200mM NaCl	200mM NaCl

Table 2. Standard evaluation score (SES) of IRRI used to assess the visual symptoms of salt toxicity.

Plant situation	**Score**
Almost all plants dead or dying	9
Complete cessation of growth; most leaves dry; some plants dying	7
Growth severely retarded, most leaves rolled; only a few are elongating	5
Nearly normal growth, but leaf tips of few leaves whitish and rolled	3
Normal growth, no leaf symptoms	1

Rate of damage was calculated as formula:

$$x = \frac{\sum(ni * a) * 100\%}{n2 * b}$$

with: x _ rate of damage of sample
a _ the evaluation score
n_i _ number of plants in the same score
b _ the highest score of plant in certain sample
n _ total number of plants in a sample

2.4. Proline measurement

Photometrical method was used for proline measurement as described by Bates et al. (1973) [16]. Proline content in samples (50mg tissue/sample) was calculated by comparison with a calibration curve which shown the relationship between proline concentration in measurement and the absorbance at 520nm wave length (A_{520}). Concentrations of 0, 25, 50, 75, 100 mM standard L-proline (Merck) were prepared to make calibration curve.

2.5. Statistical analysis

Proline content was statistical analyzed using ANOVA [17] to confirm the relationship between proline contents and NaCl concentrations in salt treatment conditions.

3. Results and Discussion

3.1. The rate of damages of soybean under salinity condition after salt acclimation

The salt tolerance of soybeans was assessed through the rate of damage caused by salt based on a standard scale of IRRI (as descripted in detail in Table 2.) [15]. The soybean DT26 plants were damaged quite much under salinity condition. The rate of damage rose when plants were irrigated with increasing saline solutions. The damage rate rapidly increased 311%, 489% and 528% when plants were suddenly watered with 50mM, 100mM and 200mM NaCl solutions, respectively in comparison with that of control plants (A0-S50, A0-S100 and A0-S200 samples in comparison to A0-S0 samples, Figure 1.). The rate of damage reduced significantly in 50mM and 100mM NaCl acclimation samples. However, 200mM NaCl was extremely hampered the development of soybean as the continuously treated with this condition (A200-S200) having 68.52% of damage rate, even higher than non acclimated plants exposed to 200mM NaCl (A0-S200 plants). The acclimation stages at 50mM and 100mM NaCl made plants more tolerance to salinity up to 200mM NaCl as rate of damage were only 49-50% in A50-S200 and A100-S200 samples.

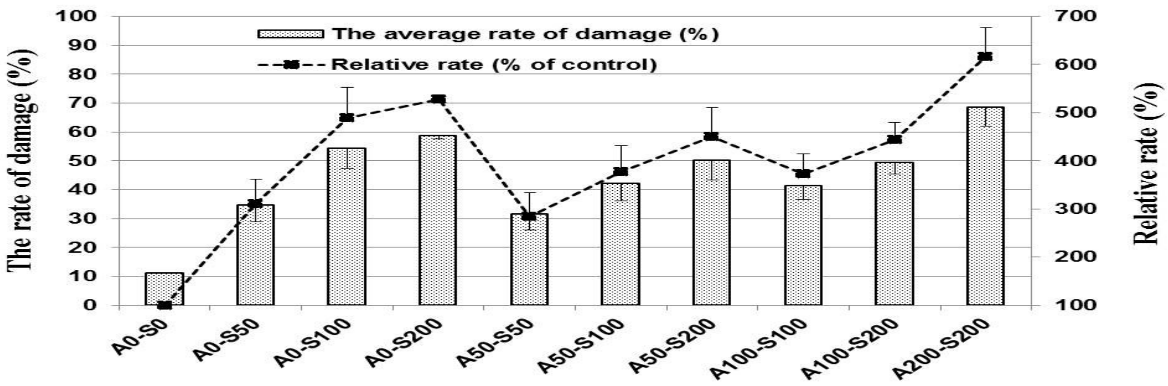

Figure 1. The average damage rate (gray bars) and the percentages of the rate (line) of soybean DT26 cultivar salt acclimated plants (A50, A100 and A200) and non-acclimated plants (A0) under salt stress (S50, S100 and S200) in comparison to control plants (A0-S0) after 14 days of treatment.

3.2. Proline accumulation differed in different tissue of soybean DT26 cultivar after salt acclimation

Once again, data showed that proline accumulted when plants exposed to salinity [1, 5, 6, 8, 11]. The seedlings in non treatment condition (A0-) synthezed more proline when were watered with high concentration of NaCl containing solutions (-S50, -S100 and –S200) than the ones continously growing in normal condition (A0-S0). Although, in all tissues the accumulation of proline was reported (Figure 2.), the changes of proline contents of different tissues were different. The proline content in roots was more than in stems and in leaves in normal condition. In salinity increase manner, the increase of proline content was observed mostly in leaves, then in stems, and least in roots. Thus, in control sample and 50mM NaCl treatmented roots proline contents were highest,following in salt treated stems and leaves, but in 100mM NaCl solution the order was from roots to leaves, to stems. Further under 200mM NaCl condition, the proline content was highest in leaves then in stems, and in roots the proline content was the least.

In comparison between non-acclimation and salt acclimation samples, proline content specially in leaves increased in acclimated samples much more than in non-acclimated samples in the same condition of salinity. For more detail, A50-S50 had from 1.3 to 2.4 and 2.7 fold of proline content more than A0-S50 in root, stem and leaf tissues, respectively. A50-S100 and A100-S100 both had nearly the same amount of proline in roots but had all about 1.5 fold of proline than A0-S100 in all other tissues. In salt stress at 200mM NaCl , the acclimated plants in 50mM and 100mM NaCl accumulated more proline than non-acclimates, but in higher NaCl concentration of 200mM during acclimation the proline content was somehow equal to the plants that were treated with 200mM NaCl without pre-trained. The proline content in leaves of A200-S200 was even less than of A0-S200. A50-S200 and A100-S200 still showed higher concentration of proline than non-acclimated plants. However, the tissue dependent proline content under high salinity conditions like that was not very clear.

In general, during acclimation the soybean DT26 cultivar tissues induced proline accumulation; acclimation stage inhibited the damage of plants in salinity and affected the biosynthesis of proline in further salt stress. However, 50mM and 100mM of NaCl treatments were active more or less the same salt tolerance in soybean DT26 cultivar.

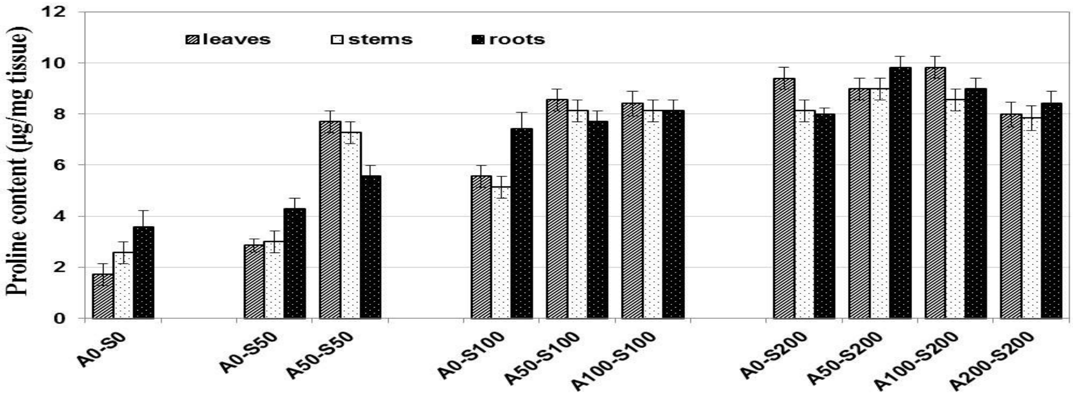

Figure 2. The proline contents in leaves (dash bars), stems (black dotted white bars) and roots (white dotted black bars) of soybean DT26 cultivar salt acclimated plants (A50, A100 and A200), non-acclimated plants (A0) under salt stress (S50, S100 and S200) and control plants (A0-S0) after 14 days of treatment.

Analysis of variance (ANOVA) was done to analyze the differences among the types of samples [17]. The analysis confirmed that proline contents depend on the salt concentration of treatment and differ from acclimation to non-acclimation with probability value $p < 0.05$ and level of confidence 95%.

4. Concluding remarks

Proline sometimes considered as one of the potential biochemical indicators of salt tolerance in plant was evaluated in soybean DT26 cultivar during salt acclimation in this study. Salt acclimation obviously reduced the damage of soybean under salinity. During acclimation DT26 soybean increased its proline accumulation which maybe in turn induced the tolerance of soybean plants. Although the accumulation of proline was mainly in roots during the first exposure to salinity it actually seems that the content of proline increased more in leaves after acclimation.

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Đánh giá hàm lượng proline ở các mô cây đậu tương (*Glycine max* [L.] Merr.) giống DT26 trong quá trình tập chống chịu mặn

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Tóm tắt: Mặn ức chế sự phát triển của thực vật. Đậu tương (*Glycine max* [L.] Merr.) là loại cây tương đối mẫn cảm với mặn, các đặc tính nông học và cả sự hình thành nốt sần ở rễ cây đều bị ức chế bởi độ mặn cao, sản lượng suy giảm đáng kể. Đậu tương có nhiều cơ chế để chống chịu mặn. Một trong số đó là tăng áp suất thẩm thấu của các tế bào. Proline có vai trò quan trọng trong cơ chế chống chịu mặn của cây. Tuy nhiên, những hiểu biết về cơ chế tập chống chịu, là khả năng tăng cường chống chịu mặn sau khi cây đã được tiếp xúc với điều kiện mặn ở mức thấp trước đó, còn hạn chế. Trong nghiên cứu này, sự thay đổi về hàm lượng proline trong quá trình tập chống chịu mặn ở giai đoạn nảy mầm của đậu tương giống DT26 được xác định. So sánh hàm lượng proline của các cây đã được tập chống chịu và cây chưa được tập chống chịu sẽ cho thấy vai trò của proline trong việc tăng cường tính chống chịu mặn. Quả thật, các kết quả cho thấy sự tăng sinh tổng hợp proline trong quá trình tập chống chịu và sự gia tăng này có khác biệt giữa các loại mô khác nhau của cây đậu tương.

Từ khóa: Proline, tập chống chịu mặn, tính chống chịu mặn, đậu tương, *Glycine max*, giống DT26.