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Changes in Soluble Sugars and Proline in Seedlings of A local Wheat Cultivar (Doha) Due to the Salt Stress and Temperature

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التغيرات في السكريات الذائبة والبرولين في بادرات الصنف المحلي للقمح (دوحة) نتيجة للاجهاد الملحي والحرارة

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درست عملية الانبات والتغيرات في السكريات الذائبة والبرولين مع الاجهاد الملحي تحت تأثير ثلاث درجات حرارية في الحبوب النابتة في صنف القمح المحلي (دوحة). أثبت هذا الصنف بأنه مقاوم للاجهاد الملحي خلال الانبات عند درجتي الحرارة 15 و 20 مئوي بالمقارنة مع درجة الحرارة 25 مئوي. وقد تعزز ذلك من خلال التراكم الكبير للكلوريد في البادرات النامية عند هذه الدرجة مقارنة مع الدرجات الحرارية المنخفضة مع زيادة تركيز كلوريد الصوديوم في محيط الحبوب. هذا فضلا عن أن درجة حرارة 25 مئوي قد تسبب هبوطا اضافيا في قيمة الجهد المائي للمحاليل والتي قد تفسر التأثير الازموزي للاملاح.

لقد تراكم البرولين لدرجة كبيرة في البادرات عند تعرض الحبوب لمستويات مختلفة من الاجهاد الملحي، وكانت العلاقة خطية بين تراكم البرولين وزيادة تركيز كلوريد الصوديوم. وان زيادة درجة الحرارة في الحاضنة قد سببت زيادة كبيرة في تركيز البرولين في الحبوب النابتة خصوصا عند مستويات الملوحة العالية. ان محتوى السكريات الذائبة (TRV) ومكوناته من السكريات الاحادية والثنائية (السكروز) قد اضمحلت في البادرات النامية المعرضة لمستويات مختلفة من الملح. كما ان درجات الحرارة الأعلى من 15 مئوي قد سببت في زيادة تكوين مستويات كبيرة من السكر الذائب عند كافة مستويات الملوحة. وهذا قد يعني سرعة تحلل النشا المخزون في اندوسبرم الحبوب بارتفاع درجة الحرارة في الحاضنة. كذلك فان هبوط TRV نتيجة للاجهاد الملحي قد تزامن مع تراكم كبير للبرولين، كما تضمنت المناقشة المصادر المحتملة للبرولين في بادرات القمح خلال عملية الانبات.

Key words: *Germination, Proline, Salt stress, Soluble sugars, Wheat*.

ABSTRACT

Germination of grains, and changes in the soluble sugars and proline due to salt stress under three temperatures in the germinating grains, of the local wheat cultivar (Doha) were studied. This cultivar proved resistant to salt stress during germination at both 15 and 20 °C as compared to 25 °C. In support to the above finding, chloride accumulated considerably in the wheat seedlings as sodium chloride increased around the grains at 25 °C as compared to that accumulated at lower temperatures. Moreover, high temperatures may cause further lowering in the water potential of salt solutions which might explain the osmotic effect of salts. Proline accumulated substantially in the seedlings exposed to various levels of salt stress, and its accumulation was linear with the increase in the concentration of NaCl. Temperature rise in the growth chamber caused a considerable increase in the concentration of proline in the germinating grains especially at high salt levels. Soluble sugars (TRV) and their components (DRV and sucrose) diminished in the growing seedlings exposed to various levels of salt stress. Temperatures above 15 °C caused significant sugar formation under all salt levels, which means that the degradation of starch stored in the endosperm of grains was accelerated with the rise in temperature in the growth chamber. Reduction in the TRV due to salt stress was accompanied with a considerable accumulation of proline. The possible sources of the accumulated proline in the seedlings of the wheat plants during germination were discussed.

1. Introduction

The adverse effect of the extreme environmental stresses on the physiological and biochemical aspects in various plants are well documented **[1, 2, 3, 4, 5, 6]**. However, information about the response of plants living in the State of Qatar to salt stress and high temperatures are scarce **[7]**. Metabolic changes due to these stresses may lead to deleterious alterations in the germination, vegetative growth and productivity of many plants such as wheat, barley and fenugreek **[8, 9, 10, 11]**. These changes include various aspects of physiology and biochemistry such as reduction in the growth processes, osmotic adjustment, hormonal balance, and the metabolism of nucleic acids, proteins and lipid **[4, 12]**. Solutes such as inorganic ions, soluble sugars, proline and glycinebeataine have proved to be the most important solutes contributing in the process of osmotic adjustment due to water stress and salinity **[4, 6, 8, 13, 14, 15, 16]**. During the period between seed germination and the seedling growth stage, plants are dependent on the reserve substances that are stored in their seeds. In the present study the changes in the soluble sugars and proline during germination of grains of the local wheat cultivar (Doha) due to NaCl stress under three temperature regimes were investigated.

2. Materials and Methods

2.1 Plant material:

Grains of the local wheat (*Triticum aestivum* L.) cultivar (Doha) were kindly obtained from the Department of Field Crop Research, Administration of Agriculture and Water Research, Ministry of Municipality and Agriculture. Viability test for these grains showed that 95% of these grains were viable. Before the germination test, healthy uniform grains were sterilized by 10 % Clorox for 2-3 minutes, thoroughly rinsed with distilled water and were left to dry.

2.2 Preparation of salt solutions:

Five salt levels (0, 10, 20, 30 and 40 dSm^{-1}) were used in the study. These levels were prepared from a standard curve of sodium chloride concentrations (%) versus the electrical conductivity (dSm^{-1}) of these solutions using a conductivity meter (model 4200 waterproof).

2.3 Germination studies:

Lots of 25 wheat grains (Doha cultivar) were placed in covered Petri dishes (11 cm diameter) upon 2 layers of filter paper, and moistened either with 10 ml distilled water (Control) or the salt solution. An extra filter paper was placed on the grains inside each Petri dish. All treatments were replicated 4 times. Petri dishes were placed in a dark growth chamber (Sanyo, Versalite Environmental Test Chamber, MLR - 350) at three temperatures (15, 20, and 25 °C) for 5 days. The following measurements were carried out for the germinated grains:

- The percentage germination was determined after 5 days of treatments.

- The radicles mean length of wheat seedlings were determined (measuring the length of radicles of 5 germinated grains in each replicate).
- Water absorption by germinating grains was determined in each replicate (gwater / 100 g grains) according the following equation:

$$\text{g water / 100 g grains} = \frac{\text{weight of absorbed water}}{\text{initial weight}} \times 100$$

2.4 Chloride determination:

Germinated grains were placed in an oven at 85 ° C for 3 – 4 days, and the plant materials were ground using (Ultra Centrigugal Mill, Zm 100, Retsch Gmbh, Germany). Dry digestion method using Muffle Furnace (Barnstead International, Type 47900 & 48000) was adopted followed by titration with silver nitrate according to the procedure described by Chapman and Pratt **[17]**.

2.5 Proline Determination:

For proline determination, the method described by Bates *et al.* **[18]** was adopted using spectrophotometer (Jenway model 6405 UV/VIS). The concentration of proline was calculated on the fresh weight basis using a standard curve.

2.6 Estimation of soluble sugars:

100 mg of oven dry and ground plant material was extracted with 5 ml of borate buffer (28.63 g. boric acid + 29.8 g. KCl + 3.5 g. NaOH in a liter of hot distilled water). The mixture was left to stand for 24 hrs, and the extract was centrifuged and filtered. The filtrate was used for the determination of the direct reducing value, DRV (which includes all free monosaccharides), and the total reducing value, TRV (which includes all soluble sugars). The procedure as described by Nelson **[19]** and successfully followed by others **[10, 16]** was used.

For the **TRV estimation,** one ml of the borate extract was added to 0.5 ml invertase (1 %) and incubated at 37 ° C for 30 minutes. After the sucrose digested by invertase enzyme, TRV was determined and the sucrose was estimated according to Nelson **[10]** formula: **Sucrose = (TRV – DRV) * 0.95 [10].** The determination of all soluble sugars was in terms of glucose from a prepared standard curve.

3. Results

3.1 Germination study:

The data shown in Figure (1) clearly illustrates that increasing salt concentration caused great reduction in the germination percentages of grains of the wheat cultivar Doha, and that the effect of salt was highly significant at the temperatures studied ($p < 0.001$). Grains of the cultivar Doha showed resistance to salt stress during germination at both 15 and 20 ºC as compared to 25 ºC. A 50 % reduction in the germination percentage was caused by the electrical conductivity (EC) of the sodium chloride solutions above 40 dSm^{-1} at both 15 and 20 ºC , whereas a salt level of less than 30 dSm^{-1} caused 50 % reduction in the germination percentage at 25 ºC. It is very probable that increase in temperature may lower the water potential of the same salt level which would then decrease the germination percentage by osmotic stress.

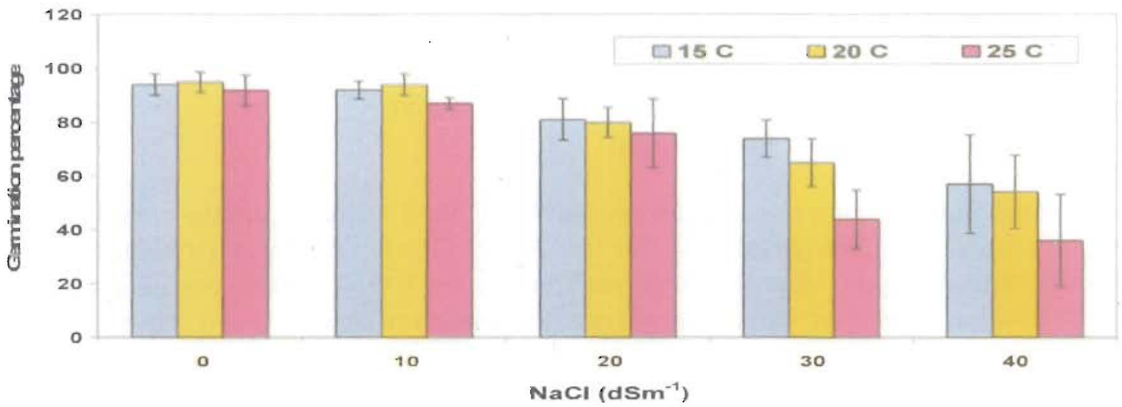

Figure (1). The effect of salt stress and temperature on the germination percentage of grains of the wheat cultivar Doha after 5 days of treatment (vertical bars indicate standard deviation).

Growth of seedlings, in terms of length of radicles, showed considerable reduction ($p < 0.001$) as the EC of the salt solution increased around the grains media (Table 1). The data revealed that the growth of radicles was very sensitive to salt stress, since 50 % reduction in the length of these radicles was observed at salt levels around 10 dSm^{-1}.

In spite of the great reduction in the germination percentages at 25 °C especially at high salinity levels, it seems, however, that this temperature had a considerable promotive effect on the growth of radicles ($p < 0.001$) after germination. Moreover, salt stress imposed by NaCl could have an osmotic effect on the growth of seedling in addition to the specific ionic effect; since water absorption showed a gradual decrease with increase salt concentration in the root environment at the studied temperatures (Table 2). The temperatures of 20 and 25 °C significantly favored water absorption at low salinity levels as compared to 15 °C ($p < 0.001$), but there were no clear differences between water contents at the three temperatures at high salinity levels.

Table (1). The effect of salt stress and temperature on the mean length of radicles (mm / radicle) of germinated grains of the wheat cultivar Doha after 5 days of treatment.

NaCl dSm^{-1}	Temperature °C		
	15± S.D.	20± S.D.	25± S.D.
Control	42.0 ± 2.5	55.0 ± 12.9	88.8 ± 6.1
10	15.7 ± 1.0	33.5 ± 3.4	35.8 ± 3.7
20	8.2 ± 1.0	17.0 ± 0.8	20.1 ± 2.0
30	2.0 ± 0.8	5.9 ± 1.0	9.1 ± 4.1
40	* Not measurable	* Not measurable	* Not measurable
Temperature	P < 0.001		
Salt stress	P < 0.001		
Interaction	P < 0.001		

*** mean radicle lengths about 1 mm**

Table (2). The effect of salt stress and temperature on the absorption of water (g water / 100 g grains) by seedlings of the wheat cultivar Doha after 5 days of treatment.

NaCl dSm^{-1}	Temperature °C		
	15 ± S.D.	20 ± S.D.	25 ± S.D.
Control	102.7 ± 22.9	140.7 ± 30.3	205.6 ± 23.3
10	77.3 ± 10.8	124.1 ± 7.3	118.9 ± 22.1
20	56.5 ± 1.5	67.8 ± 4.8	58.3 ± 13.7
30	42.8 ± 1.8	45.3 ± 3.4	44.8 ± 4.2
40	40.2 ± 2.1	43.8 ± 1.9	40.7 ± 2.3
Temperature Salt stress Interaction	$P < 0.001$ $P < 0.001$ $P < 0.001$		

3.2 Chloride accumulation:

Chloride accumulated considerably in the germinated seedlings exposed to salt stress imposed by NaCl (Figure 2), and the accumulation was linear with the increase in the concentration of the salt. Equally increase in the temperature caused significant accumulation of chloride in the growing seedlings at all salinity levels ($p < 0.001$).

However, the accumulation at 25 °C did not show any adverse effect on the growth of the radicles of the growing seedlings. On the other hand, chloride accumulation might have had a great influence on the decrease of the water potential of the germinating grains (water potential becomes more negative) thereby contributing in the osmotic adjustment in seedling tissues.

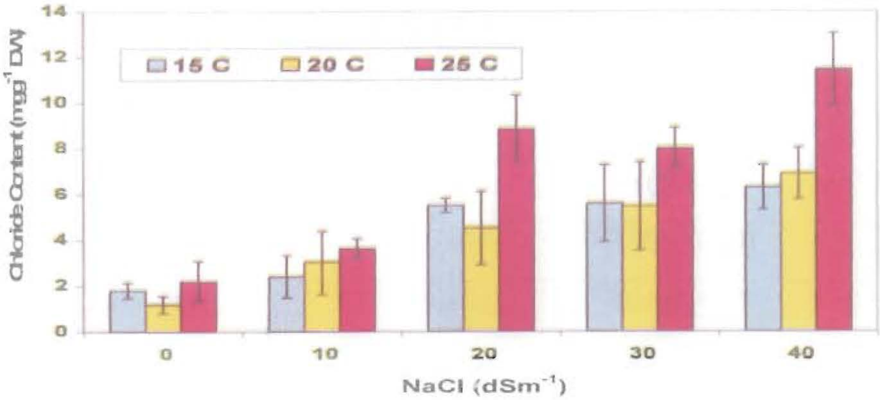

Figure (2). The effect of salt stress (imposed by NaCl) and temperature on the chloride accumulation (mg g^{-1} DW) in the seedlings of the wheat cultivar Doha after 5 days of treatment. (vertical bars indicate standard deviation)

3.3 Accumulation of proline and sugar in seedlings:

Proline accumulated substantially ($p< 0.001$) in the seedlings exposed to various levels of salt stress as shown in Table (3). There was a linear relationship between accumulation and the increase in the NaCl concentration. It seems that a rise in the temperature in the growth chamber causes considerable increase in the concentration of proline in germinating grains especially at high salt levels.

Table (3). The effect of salt stress and temperature on proline accumulation (µg g^{-1} FW) in the seedlings of the wheat cultivar Doha after 5 days of treatment.

NaCl dSm^{-1}	Temperature °C		
	15 ± S.D.	20 ± S.D.	25 ± S.D.
Control	145.2 ±14.3	147.4 ±9.3	142.7 ±42.6
10	236.2 ±12.9	309.8 ±9.5	209.7 ±14.4
20	301.2 ±33.2	351.1 ±16.6	408.4 ±8.8
30	331.1 ±14.3	431.2 ±2.4	411.5 ±68.3
40	383.3 ±11.4	484.5 ±15.4	462.7 ±33.6
Temperature Salt stress Interaction	$P < 0.001$ $P < 0.001$ $P < 0.01$		

The data shown in Figure (3) revealed that the soluble sugars (TRV) diminished in the growing seedlings exposed to various levels of salt stress. It is interesting to mention that increasing temperature caused significant sugar formation which means that the degradation of starch stored in the endosperm of the grains was accelerated with the increase in the temperature in the growth chamber.

Fig. 3 (A) TRV

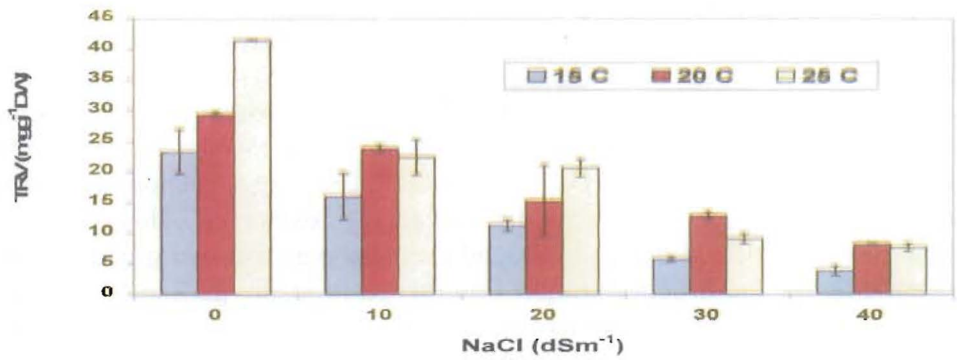

Fig. 3 (B) Proline content

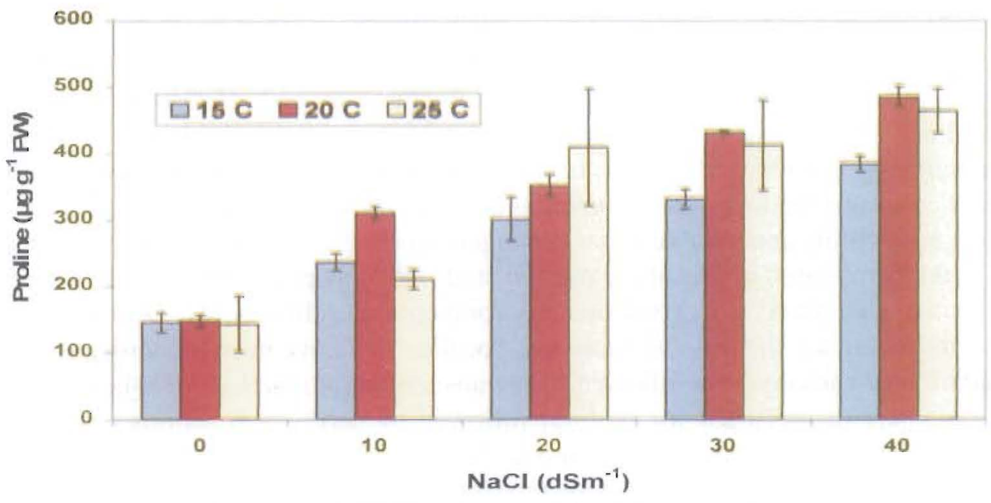

Figure (3) The effect of salt stress and temperature on TRV (mg g^{-1}DW) and proline (μg g^{-1} FW) content in the seedlings of the wheat cultivar Doha after 5 days of treatment. (vertical bars indicate standard deviation)

on the Other hand, Such reduction in the TRV was accompanied with the considerable accumulation of proline (Figure. 3). Moreover, the great reduction in the contents of both DRV and sucrose has contributed effectively in the magnitude of TRV with salt stress at the studied temperatures (Table 4). It seems that both DRV content and sucrose concentration were highly correlated. Linear regression was fitted between DRV as independent variable (X) with sucrose as dependent variable (Y). The following linear equation was obtained:

Sucrose (mg g^{-1} DW) = 0.747 + 0.861 x DRV (mg g^{1}DW), and the Correlation Coefficient (r) of this relation was (r = 0.89). This means that monosaccharide and sucrose have had similar effect in their contribution in determining the value of TRV.

4. Discussion

Reduction in the germination percentages of grains (or seeds) of most crop plants is a common response when exposed to salt stress higher than 10 dSm^{-1} **[20, 21]**. In fact sodium chloride affects germination in two main ways: (a) osmotic effects by decreasing water uptake, and (b) toxic effect (specific ionic effect) of the accumulating Na and Cl ions **[12, 22, 23]**. The results obtained in the present study confirmed the osmotic effect of sodium chloride since water absorption was decreased substantially with salt stress. Also, the considerable accumulated Cl^{-1} ions in the seedlings as the concentration of sodium chloride was increased around grains may have toxic effect on the growth of the embryos of grains' **[24, 25]**. Both osmotic and ionic effects cause great reduction in the growth processes **[26]**, thereby decreasing the germination percentages and reducing the growth of radicles of seedlings **[27, 28]**. The reduction in the length of radicles is attributed to the inhibition of both cell division and cell expansion. This is in support of the finding that MI (Mitotic Index) in the radicles of the germinated grains of barley showed gradual decrease as the NaCl concentration was increased in the germination medium **[27, 29]**. Other possibilities for reduction in the growth of radicles under osmotic stress include: (a) G1 and G2 of the interphase were arrested, (b) reduction in the biosynthesis and / or acceleration of degradation of nucleic acids, and (c) disturbance in the hormonal activity **[4]**.

Increasing temperatures could have further reduction in the germination percentage with salt stress possibly due to: (a) the water potential of the salt solutions might become more negative with increase in temperature in the growth chamber, and (b) accumulation of ions such as Na^+ and Cl^- could have toxic effect of NaCl **[25, 30]**. However, chloride accumulation in the seedlings, as observed in the present study, did not cause any further reduction in the growth of radicles. On the contrary, the radicles were longer at 25 °C at all salinity levels as compared to the other two temperatures (15 and 20 °C). It is interesting to mention that the wheat cultivar Doha was developed to suit the local environmental conditions, and it could have been well adapted to such conditions.Proline and soluble sugars changed substantially with the osmotic stress imposed by NaCl in the germinated grains of the present study. Proline plays many roles in the physiology and biochemistry in plants under various environmental conditions **[4]**. During germination of grains, the following are the possible sources of proline that have been recognized:

(a) Degradation of carbohydrate followed by a transamination reaction of α-keto-glutaric acid to produce glutamate (a precursor for proline) followed by series of reactions started with kinase reaction **[31]**. However, such accumulation could be accelerated by darkness **[32]**. In fact, during dark incubation of the wheat grains, it seemed that the main source of proline came from the degradation of starch stored in the endosperm of grains.

(b) The rapid degradation of protein during germination under stress conditions could be another source of proline **[33]**. Moreover, glutamate and arginine can be formed after the degradation of the protein stored in the aleurone layer of wheat grains followed by series of reactions to build proline **[4, 34]**. In a previous study on some barley cultivars ([27], it was suggested that protein degradation under salt stress could be insufficient to explain the high concentrations of proline found in the germinating grains. Thus, during the germination stage there was a requirement for carbohydrate to provide the carbon skeleton for proline accumulation more than that released by

Table (4) The effect of salt stress and temperature on the DRV content and sucrose formation (mg g^{-1} DW) in the seedlings of the wheat cultivar Doha after 5 days of treatment.

NaCl dSm^{-1}	DRV			Sucrose		
	15 °C ± S.D.	20 °C ± S.D.	25°C ± S.D.	15 °C ± S.D.	20 °C ± S.D.	25°C ± S.D.
Control	8.93 ± 1.01	14.93 ± 1.27	21.74 ± 4.48	13.60 ± 3.02	14.00 ± 1.02	18 .70 ± 4.25
10	7.43 ± 1.33	11.60 ± 1.14	12.02 ± 0.72	8. 08 ± 2.39	11.52 ± 1.58	9.73 ± 2.49
20	4.07 ± 1.19	10.07± 3.86	9.04 ± 0.14	6.81 ± 1.52	5.13 ± 2.07	10.93 ± 1.29
30	2.93 ± 0.58	5.67 ± 0.84	5.73 ± 0.70	2.61± 0.90	6.73 ± 1.35	3.10 ± 0.17
40	2.34 ± 0.29	4.19 ± 0.90	4.50 ± 1.13	1.40 ± 0.78	3.72 ± 0.85	2.95 ± 1.65
Temperature	P< 0.001			P < 0.001		
Salt stress	P< 0.001			P < 0.01		
Interaction	P< 0.001			P < 0.01		

proteolysis to build proline **[35]**. The data of the present study showed that all soluble sugars were diminished in the germinating grains under different temperatures which were accompanied by substantial accumulation of proline. In fact proline concentration was inversely related with concentration of soluble sugars (TRV) under different temperatures (see Figure 3). Linear regression was fitted between TRV as independent variable (X) with proline as dependent variable (Y). The following linear equation was obtained:

Proline (µg g^{-1} FW) = 462.35 - 8.73* TRV (mg g^{-1}DW), and the Correlation Coefficient (r) of this relation was (r = - 0.76).

(c) It is possible that the reduction of the cell size under salt stress **[26]** could lower the osmotic potential by increasing the concentration of solutes in a limited volume which might play a role in the increasing concentration of proline **[36, 37]**.

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