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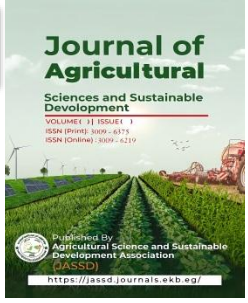

Investigate the response of vegetative growth and chemical constituents of *Agave* Sp. plants grown under saline condition

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Abstract
A pot experiment was carried out in the glasshouse of National Research Centre, Giza Dokki, Egypt during two successive seasons 2020 and 2022 to examine the influence of proline spraying treatments on irrigation by saline water on *Agave* growth parameters and chemical contents, in three sprays at concentrations (0, 100 and 200 ppm) and salt stress (0, 5000, 10000 and 20000 ppm) with the control. However, the highest value of these characters (plant height, leaves number, leaf area, fresh weight/plant and dry weight/plant) obtained at up 100 ppm proline while (shoots and roots elongation and shoot and root fresh weights) were showed the highest increments by 5000 ppm salt irrigation, as well as the lowest values of carbohydrate % (0.39, 89.77, 19.78 and 314.44) resulted from the highest-level salinity by 15000 ppm with insignificant difference in flowering, and chemical characters. In addition, the proline treatments showed insignificant effect. It caused an increase in previous vegetative measurements and chemical constituents such as osmotic pressure, protein % and electrical conductivity analysis at 100 and 200 ppm compared with control. The purpose of study is to know more about the effects of proline and salinity stress on plant development and chemical components.

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INTRODUCTION:

Agave belongs to the family: Agavaceae. It has a rosette of thick fleshy leaves. *Agave* plants are native to arid and semi-arid regions. Several cultivated of *Agave* species can perform well insufficient rainfall areas. Alcoholic, beverages, sweeteners, fibers and some specialty chemicals coming from *Agave* plants. The blue *Agave* considers economic, social and cultural importance in Mexico, because it is the raw material for the 'tequila' conduction which consider national and centenary alcoholic beverage, **Granados, (1993).** *Agave americana* has successfully adapted to climate change, edaphic conditions, it is an important natural fiber source in medicine, fructans and several commercial plantations for agro-industrial use, **Torre-Ruiz, *et al.*, (2016).** The enhancement in endogenous proline level is cumulative of its biosynthesis induction, reduction in degradation and re-allocation between the cells via Pro transport proteins, **Mansour and Ali, (2017) and Kesawat, *et al.*, (2023).**

High salt stress directly or indirectly inhibits cell division, enlargement in the growing tissues of roots, stem and leaves, may lead to leaf burn and defoliation, and reduced plant height, dry weight, number of leaves and number of flowers. The leaves die sooner in a more salt sensitive variety because salts arrive faster or because cells are unable to compartmentalize the salts in vacuoles, **Avolio and Bass (1995)** and **Sonneveld, *et al.*, (1999).** The proline induced salt tolerance in *Cakile maritima* via ornithine pathway during salt stress, **Woodrow, *et al.*, (2017) and Al Otaibi, *et al.*, (2024).**

Generally, salinity and water stress (drought) have morphological, physiological, anatomical and biochemical effects on plants' growth. Several investigators in this respect said that the deleterious effect of salinity on plants was reported by **Tiwari, *et al.*, (2010)** on cucumber, **Hameed, *et al.*, (2011)** on **wheat and Fayez and Bazaid (2014)** on barley. The physiological responses to salt stress by a variety of mechanisms including osmotic adjustment, high selectivity of K^+ over Na^+, **Messedi, *et al.*, (2016) and Andrade-Marcial, *et al.*, (2023).**

High salinity also caused osmotic damage because of the buildup of high concentrations of Na ions in plant leaves. Since Na ions entered leaves in xylem stream and were left behind as water evaporated, **Khalil, *et al.*, (2017).** The aim of this study is to investigate the response of vegetative growth and chemical constituents of *Agave* plants grown under saline conditions. Osmotic adjustment (OA) is a physiological adaptation which has drawn attention, **Li, *et al.*, (2017), Nguyen, *et al.*, (2017) and Spormann, *et al.*, (2023).**

MATERIALS AND METHODS:

Experiment was conducted on *Agave* plant during the two summer seasons of 2020-2022 at the greenhouse of the National Research Centre, Dokki, Cairo to evaluate the effect of different salt stress degrees and proline acid on photosynthetic pigments, water turgidity and osmotic adjustment pressure of the leaves. *Agave* seedlings 15 (cm) were planted during the winter season in November in pots of 30 cm^2 diameter filled with 10 kg soil clay and sand (1:1 by volume). The chemical analyses of the soil were carried out according to the method described by **Holliday, (1990) and Klute, (1986)** in Table (1). All pots received the same doses of nitrogen, phosphorus and potassium and equal amounts of organic matter. Seedlings were irrigated till emergence with tap water to avoid salinization check of seedling.

Table (1): Analysis of soil (Average of two seasons)

Appreciation	**Sample**
pH (1:2.5)	7.66
EC (dSm-1) (1:5)	0.72
OM	0%
Soluble cations (ml, milliliter equivalent / liter) mEq/L-1	
(CaCO2) Ca++	1.0
Mg++	0.8
Na+	5.6
K+	0.05
Dissolved soluble anions (mEq/L-1)	
CO3=	-
HCO3-	1.5
Cl-	2.5
SO4=	3.45
Pb	-
Soil types	
Sand	50%
Silt	0%
Clay	50%

The seedlings were subjected to salinity levels (0, 5000, 10000 and 15000 ppm) after 90 days from transplanting. Salinity stress was done by using Stroganov solution, **Stroganov, (1964).** Spraying with proline acid at the rate of (0, 100 and 200 ppm) were sprayed twice, the first after 105 days from subjected to irrigation with saline water and the second two weeks later and the control sprayed with tap water. The experiments consists of 12 treatments which were the combination of 4 levels of salinity and 3 level of proline acid. The design of the experiment was a split plot design with 6 replicates. Data were subjected to analysis of variance according to **Snedecor and Cochran (1980).** Means was compared using L.S.D. at 5 % levels of probability.

Fresh leaves will be sampled from every treatment for determination of:

1- Pigments contents (chlorophyll a, b and carotenoids (mg / g f. w.)) were determined according to **Metzner, *et al.*, (1965).**

2- Total carbohydrates (% d. w.) were determined according to the method by **Herbert, *et al.*, (1971).**

3- Proline content (m. mol / ml d. w.) was determined according to the method by **Bates, *et al.*, (1973).**

4- Relative water content (mg / g f. w.) according to **Weatherly, (1962).**

5- Osmotic pressure (atm) by using refract meter and the corresponding values were then recognized from table given by **Gusev, (1960).**

6- Protein percentage (mg / ml d. w.) Crude protein percentage was determined using the equation of **Alsmeyer, *et al.*, (1974).**

7- Electrical conductivity (ug / g f. w.) were determined according to **A.O.A.C., (1990).**

The following data was recorded:

1- Plant height (cm).

2- Stem diameter (mm).

3- Leaf area index (cm^2).

4- Number of branches / plants (no.).

5- Number of leaves / plants (no.).

6- Fresh weight of plant (g).

7- Dry weight of plant (g).

RESULTS AND DISCUSSION:

The growth parameters as affected by saline water irrigation treatments Table (2) in both seasons. Increasing the concentration of salinity from 5000 up to 15000 ppm significantly decreased the values of growth parameters (plant height, leaves number, leaf area, fresh weight/plant and dry weight/plant). The lowest values of previously mentioned parameters were obtained from the plants irrigated with the highest concentration of salinity (15000 ppm). The depressive effect on plant height by salinity might be mainly attributed to reduction in cell enlargement and division induced by salinity, also closes of stomata which reduced the supply of dioxide for photosynthesis. The decrease in fresh weight of *Agave* plants by irrigated saline water might be due to the inhibition of water absorption, distribution of mineral balance, absorption and utilization under salinity conditions, **Mazher, *et al.*, (2006).** These results are agreed with **Alam, *et al.*, (2014), Amirjani, (2015) and Torre-Ruiz, *et al.*, (2016).** While, the exposing to salinity (0-1000 mM NaCl) for ten days, **Belkheiri and Mulas (2013)** found that growth increased up to 400 mM NaCl and decreased at higher salinity stress. It is climate change may challenge the crops to adapt to rising temperatures, salinity, drought, pests, diseases, and flooding, **Roca, *et al.*, (2023).**

Salinity limited plant growth (shoots and roots elongation and shoot and root fresh weights). The inhibition in plant growth may be due to the lowering in water potential and turgidity of stressed tissues that caused internal water deficit to plants as recorded by **Ascencio-Valle, *et al.*, (2013).** However, **Munns and Tester (2008)** indicated that the inhibition in plant growth by salinity might be due to the bad effects of toxic ions mainly Na^+ and Cl^-. **Kaydan, *et al.*, (2007) and Abdul Qados, (2015)** obtained similar results, they returned such reductions in growth to decrease in water availability by plant roots, which led to the disturbance in water status of plant's tissues and metabolic processes, leading to reductions in meristematic activity and cell size. It also caused an increment in respiration rate due to the higher energy requirements. The relative contribution of K^+ to the total osmolality decreased with increased NaCl levels especially in roots. In halophytes, the involvement of Na^+ in OA has been well. In *Ricinus communis* **L, Rodrigues, *et al.*, (2014)**, showed that the salt ions reached values of 55, 65 and 69% in leaves and 33, 42 and 58% in roots when plants were respectively subjected to 50 mM, 100 mM and 150 mM NaCl treatments during 15 days, **Ronceret and Bolaños-Villegas (2024).**

Table (2) shows that growing seedlings under salinity conditions led to a reduction in total carbohydrate percentage in leaves compared to control at both seasons. The lowest values (0.39, 89.77, 19.78 and 314.44) resulted from the highest level of salinity. These results agree with those obtained by **Bernstein, *et al.*, (1972)** who stated that, they obtained reduction in total carbohydrate percentage as salinity increased might have relation to respiration processes since the free sugars were the main sugar pattern involved in the mechanism of respiration. Also, **Batanouny, *et al.*, (1988)** who mentioned that the lowered photosynthetic ability under salt stress condition was due to stomata closure, inhibition of chlorophyll synthesis, a decrease of carboxylase enzyme and due to high chlorophyll activity. The plant remobilizes its nitrogen reserve by the degradation of nitrogenous compounds (Rocha da, *et al.*, 2012). The genetically modified organisms, have shown promise in mitigating the impact of drought and salinity stress, **Raza, *et al.*, (2023).**

Table (2): Effect of salinity on growth and some chemical constituents of *Agave* plants (Average of two seasons).

Measurements / Treatments	Plant height (cm)	Leaves number (no.)	Leaf area (cm^2)	Fresh weight of plant (g)	Dry weight of plant (g)	Osmotic pressure (atm)	Total carbohydrate Percentage (% d. w.)	Protein % (mg / ml d. w.)	Electrical Conductivity (ug / g f. w.)
0 ppm	46.89	10.56	173.22	2.05	12.17	27.12	0.66	123.67	434.56
5000 ppm	46.00	09.11	163.33	2.04	09.33	26.30	0.54	103.27	430.78
10000 ppm	46.00	07.78	154.11	1.73	09.06	21.95	0.52	099.47	367.11
15000 ppm	43.11	07.11	147.00	1.49	06.87	19.78	0.39	089.77	314.44
L.S.D. 0.05	04.90	00.89	041.92	0.72	00.06	00.06	7.94	000.09	152.19

Table (3) in both seasons, increasing the concentration of proline from 100 to 200 significantly decreased the values of growth parameters and chemical constituents in most cases. As for the effect of proline application on carbohydrate percentage, proline decreased carbohydrate % with increasing proline up to 200 ppm. **Ackerson, *et al.*, (1984)** argued that cellular osmotic adjustment occurs in response to stress via an active or passive accumulation of solutes. It has been assumed that stress enhanced the production of proline, which causes osmotic adjustment, **Al- Bahrany, (1994).** The data of interaction effect among the two studied factors (salinity level plus proline spray) on proline content show the high proline values were found when using 15000 ppm salinity with untreated proline treatment. The regulatory effect of amino acids application may be through their effect on gibberellins biosynthesis, **Walter and Nawacki, (1978).** The amino acids could play a role in plant metabolism and protein assimilation, which was important for cell formation and consequently increase in fresh and dry weights. Such the operation of biosynthetic pathways might due to differences in nutritional requirement such as nitrogen (N) nutrition, **Abd Elgawad, *et al.*, (2015).** Increasing salinity stress modifies soil texture, causing decreased porosity, which causes reduced water uptake by plants, **Lu and Fricke (2023).**

Interaction effect in Table (4) indicated that the highest values of all growth parameters were obtained due to the use of (5000 ppm salinity + 100 ppm proline, 10000 ppm salinity + 100 ppm proline, 15000 ppm salinity + 100 ppm proline, 5000 ppm salinity + 200 ppm proline, 10000 ppm salinity + 200 ppm proline and 15000 ppm salinity + 200 ppm proline) respectively, compared with other treatments. The δ-OAT showed to participate in proline biosynthesis in several plants such as Cashew leaves, submitted to salt **(Rocha da, *et al.*, 2012).** The impact of salinity on plant productivity escalates, leading to economic losses and societal effects, **Atta, *et al.*, (2023).**

Observed decrease in growth parameters due to the use of combined treatment of both amino acids compared with single treatment under different salinity levels. **Yamada, *et al.*, (2005) and Abd El-Samad, *et al.*, (2011)** confirmed that osmotic and specific ion effects were the most frequent mechanism by which salinity stress inhibited plant growth. Such effects resulted by reducing water uptake due to nutritional imbalance caused by element antagonism or toxicity. The regulatory effect of amino acids application may be through their effect on gibberellins biosynthesis **Walter and Nawacki, (1978).** In addition, amino acids could play an important role in plant metabolism and protein assimilation, which was important for cell formation, and consequently increase in fresh and dry weights. The ornithine pathway is predominant in leguminous plant for biosynthesis and accumulation of proline in stressed plants, whereas grass species accumulated proline via glutamate pathway **(Mansour and Ali, 2017).** In the saline environment, the application of Zn is known to enhance salinity tolerance and stimulate proline metabolism, **Mushtaq, *et al.*, (2023) and Acharya, *et al.*, (2023).**

Table (3): Effect of proline on growth and some chemical constituents of *Agave* plants (Average of two seasons).

Measurements / Treatments	Plant height (cm)	Leaves number (no.)	Leaf area (cm^2)	Fresh weight of plant (g)	Dry weight of plant (g)	Osmotic pressure (atm)	Total carbohydrate Percentage (% d. w.)	Protein % (mg / ml d. w.)	Electrical Conductivity (ug / g f. w.)
0 ppm	46.17	9.42	188.25	1.86	11.07	30.03	0.56	109.18	392.92
100 ppm	45.75	8.42	160.92	1.83	09.52	23.14	0.51	102.15	388.25
200 ppm	44.58	8.08	129.08	1.79	07.49	18.20	0.50	100.80	379.00
L.S.D. 0.05	04.24	0.69	036.31	0.62	00.05	00.05	6.87	000.08	131.80

Table (4): Interaction effect between salinity and proline on growth and chemical constituents of *Agave* plants (Average of two seasons).

Measurements / Treatments	Plant height (cm)	Leaves number (no.)	Leaf area (cm^2)	Fresh weight of plant (g)	Dry weight of plant (g)	Osmotic pressure (atm)	Total carbohydrate Percentage (% d. w.)	Protein % (mg / ml d. w.)	Electrical Conductivity (ug / g f. w.)
0 ppm	47.67	11.00	153.33	1.53	322.66	08.16	37.77	0.495	098.40
5000 ppm	40.00	09.00	173.67	2.08	439.61	06.81	24.83	0.608	081.80
10000 ppm	41.67	07.33	114.00	1.60	337.46	12.21	16.30	0.449	118.20
15000 ppm	42.33	07.00	090.00	1.98	418.47	09.39	19.43	0.466	092.20
100 ppm+5000 ppm	49.00	07.33	172.67	1.91	402.97	12.90	20.43	0.402	088.70
100 ppm+10000 ppm	46.67	07.00	199.67	2.27	480.47	14.21	25.98	0.292	088.40
100 ppm+15000 ppm	45.67	07.00	160.00	2.39	504.42	02.26	35.50	0.486	091.80
200 ppm+5000 ppm	49.00	08.67	178.00	1.81	381.84	09.18	32.50	0.506	176.43
200 ppm+10000 ppm	47.33	07.67	152.00	1.91	404.38	09.18	13.35	0.614	102.40
200 ppm+15000 ppm	42.67	07.33	113.00	1.27	268.42	10.14	27.40	0.782	126.20
100 ppm	50.67	12.67	228.67	1.54	326.32	09.18	14.78	0.491	089.80
200 ppm	47.33	11.67	178.00	1.64	347.32	08.67	17.17	0.694	093.83
L.S.D. 0.05	08.49	01.38	072.61	1.25	263.61	00.11	00.10	4.350	000.15

Some photos from the field of experiment

CONCLUSION AND RECOMMENDATIONS:

It is concluded that accumulation of proline is a tool of defense with high levels of salt and non-uptake water. In addition, they represent chemical components of plants and reaction center complexes of storage and tolerance organisms. It is considered as an induce to long survive; moreover, it exhibits anti-drought and anti-parent. It can be recommended to grow the agave plant under environmental conditions with increased salinity by spraying with proline or without spraying with proline, as it showed a strong growth response under high salinity conditions.

REFERENCES:

A. O. A. C. (1990). Official methods of analysis of the association of official agricultural chemist 13th edition. Washington, D.C., U.S.A.

Abd Elgawad, H.; D. G. De Vos Zinta; M. A. Domagalska; G. T. S. Beemster and H. Asard (2015). Grassland species differentially regulate proline concentrations under future climate conditions: an integrated biochemical and modelling approach. New Phytol., 208: 354–369.

Abd El-Samad, H.; M. A. K. Shaddad and N. Barakat (2011). Improvement of plants salt tolerance by exogenous application of amino acids. J. Medic. Plants Res., 5 (24): 5692-5699.

Abdul Qados, A. M. S. (2015). Effects of salicylic acid on growth, yield and chemical contents of pepper (Capsicum annuum L) plants grown under salt stress conditions. Inter. J. Agric. Crop Sci., 8 (2): 107–113.

Acharya, B. R.; S. P. Gill; A. Kaundal and D. Sandhu (2024). Strategies for combating plant salinity stress: the potential of plant growth-promoting microorganisms. Front. Plant Sci., 15: 1406913.

Ackerson, R. C.; Havelka, U. D. and M. G. Boyle (1984). CO2-Enrichment Effects on Soybean Physiology. II. Effects of

Stage-Specific CO2 Exposure1, Crop Sci., 30 (24), 1150-1154.

Al Otaibi, A. F.; A. A. Sameera and K. A. M. Abo-Elyousr (2024). The Influence of Salinity on Plant Growth and Amendment Strategies. Sohag J. Sci., 9 (3), 261-267.

Alam, M.; D. Akram; E. Sharmin; F. Zafar and S. Ahmad (2014). Vegetable Oil Based Eco-Friendly Coating Materials: A Review Article. Arab. J. Chem., 7 (4), 469-479.

Al-Bahrany, A. M. (1994). Influence of salinity on free proline accumulation, total RNA content and some minerals (K, Na, Ca, Mg and N) in pepper, Capsicum annuum L. Annals Agric. Sci., Ain-Shams Univ., Cairo, Egypt, 39 (2), 699-707.

Alsmeyer, R. H.; A. E. Cunningham and M. L. Happich (1974). Equations predict PER from amino acid analysis. Food Techn., 28 (7): 34-40.

Amirjani, M. R. (2015). Effect of salinity stress on seed germination and antioxidative defense system of Catharanthus roseus. ARPN J. Agric. Bio. Sci., 10 (5): 163-171.

Andrade-Marcial, M.; E. Ruíz-May; J. M. Elizalde-Contreras; N. Pacheco; E. Herrera-Pool and C. De-la-Peña (2023). Proteome of Agave angustifolia Haw.: Uncovering metabolic alterations, over-accumulation of amino acids, and compensatory pathways in chloroplast-deficient albino plantlets. Plant Physio. Biochem., Volume 201, 107902.

Ascencio-Valle, H. F.; H. Fujiyama; A. Orduño-Cruz; T. Endo and J. Á. Larrinaga-Mayoral (2013). Differential responses of Jatropha Species on growth and physiological parameters to salinity stress at seedlings plant stage. Comm. Soil Sci. Plant Ana. Tandfon.com/doi/pdf, online, August.

Atta, K.; S. Mondal; S. Gorai; A. P. Singh; A. Kumari and T. Ghosh (2023). Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. Front. Plant Sci., 14.

Avolio, B. J. and B. M. Bass (1995). Individual Consideration Viewed at Multiple Levels of Analysis: A Multi-Level Framework for Examining the Diffusion of Transformational Leadership. Leadership Quarterly, 6, 199-218.

Batanouny, K. H.; M. M. Hussein and M. S. A. Abo EL-Kheir (1988). Response of Zea mays to temporal variation of irrigation and salinity under farm conditions in the Nile Delta, Egypt. Proc. Inter. Conf. Plant Growth, Droug. Salin. Arab Region, Cairo Univ. Egypt, December 3-7, pp: 189–204.

Bates, L. S.; R. P. Waldren and I. D. Teare (1973). Rapid Determination of Free Proline for Water Stress Studies. Plant Soil, 39: 205–207.

Belkheiri, O. and M. Mulas (2013). The effects of salt stress on growth, water relations and ion accumulation in two halophyte Atriplex species. Environ. Exp. Bot., 86: 17–28.

Bernstein, L.; L. E. Francois and R. A. Clark (1972). Salt tolerance of ornamental shrubs and ground covers. J. Amer. Soc. Hort. Sci., 97: 550-556.

Fayez, K. A. and S. A. Bazaid (2014). Improving Drought and Salinity Tolerance in Barley by Application of Salicylic Acid and Potassium Nitrate. J. Saudi Soc. Agric. Sci., 13, 45-55.

Granados, S. D. (1993). Los Agaves en México. Universid. Autóno. Chapingo., México, Texcoco, 252, pp. 1-35.

Gusev, N. A. (1960). "Some methods for studying plant water relations.". Akad-Nauke S.S.S.R., Leningrad.

Hameed, A.; N. Bibi; J. Akhter and N. Iqbal (2011). Differential changes in antioxidants, proteases and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physio. Biochem., PPB, 49 (2):178-185.

Herbert, D.; P. J. Phipps and R. E. Strange (1971). Chemical Analysis of Microbial Cells. Meth. Microbe., 5, 209-344.

Holliday, V. T. (1990) and Klute, A. Ed. (1986). Methods of soil analysis, part 1, physical and mineralogical methods (2nd edition), part 2, chemical and microbiological properties. Ameri. Soc. Agron., Agron. Monogra., Madison, Wisconsin, U.S.A., 9 (1), pp. 1188, Geoarch. Inter. J., 5, 87-89.

Kaydan, D.; Y. Mehmet and O. Neşe (2007). Effects of Salicylic Acid on the Growth and Some Physiological Characters in Salt Stressed Wheat (Triticum aestivum L.). Tarim Bilimleri Dergisi, 13 (2): 114-119.

Kesawat, M. S.; N. Satheesh; B. S. Kherawat; A. Kumar; H. U. Kim; S. M. Chung and M. Kumar (2023). Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—Current perspectives and future directions. Plants, 12 (4), 864.

Khalil, E. Soha; S. A. Metwally and Bedour H. Abou Leila1 (2017). Changes Induced in Some Physiological, Biochemical and Anatomical Characteristics of Jatropha curcas L. Plant by Exogenous Application of Osmo-Regulators Under Saline Irrigation Conditions. (RJPBCS) Research Journal of Pharmaceutical, Biological and Chemical Sciences, November–December 8 (6): pp. 96-111.

Li, Z.; D. Peng; X. Zhang; Y. Peng; M. Chen; X. Ma; L. Huang and Y. Yan (2017). Na+ induces the tolerance to water stress in white clover associated with osmotic adjustment and aquaporins-mediated water transport and balance in root and leaf. Environ. Exp. Bot., 144: 11–24.

Lu, Y. and W. Fricke (2023). Salt stress—regulation of root water uptake in a whole-plant and diurnal context. Int. J. Mol. Sci., 24, 8070.

Mansour, M. M. F. and E. F. Ali (2017). Evaluation of proline functions in saline conditions. Phytochem., 140: 52–68.

Mazher, Azza A. A.; A. A. Rawya Eid and G. Nahed Abdel-Aziz (2006). Effects of microbien under salt stress on nodulation, growth and chemical constituents of Sesbania aegyptiaca in sandy soil. Bull. Nat. Res. Centre, Cairo, 31 (3): 245-265.

Messedi, D.; F. Farhani; K. B. Hamed; N. Trabelsi; R. Ksouri; H.-u-R. Athar and

C. Abdelly (2016). Highlighting the mechanisms by which proline can confer tolerance to salt stress in Cakile maritima. Pak. J. Bot., 48: 417–427.

Metzner, H.; H. Rau and H. Senger (1965). Determination of photosynthetic pigment mangel - Mutanten von Chlorella, Ultersuchungen zur synchronisier barkeit einzelner. Planta, 65 (2): 186-194.

Munns, R. and M. Tester (2008). Mechanisms of Salinity Tolerance. Annual Review Plant Bio., 59: 651- 681.

Mushtaq, N. U.; K. M. Alghamdi; S. Saleem; I. Tahir; A. Bahieldin and B. Henrissat (2023). Exogenous zinc mitigates salinity stress by stimulating proline metabolism in proso millet (Panicum miliaceum L.). Front. Plant Sci., 14.

Nguyen, H. T.; P. Meir; L. Sack; J. R. Evans; R. S. Oliveira and M. C. Ball (2017). Leaf water storage increases with salinity and aridity in the mangrove Avicennia marina: integration of leaf structure, osmotic adjustment and access to multiple water sources. Plant Cell Environ., 40: 1576–1591.

Raza, A.; J. Tabassum; A. Z. Fakhar; R. Sharif; H. Chen and C. Zhang (2023). Smart reprograming of plants against salinity stress using modern biotechnological tools. Crit. Rev. Biotech., 43, 1035–1062.

Roca, M. M.; J. Falck- Zepeda and P. Paes-Andrade (2023). Cisgenics and genome editing or second-generation biotechnologies: A Latin America and Caribbean perspective. In Chaurasia A. & C. Kole (Eds.), Cisgenic crops: Safe., Legal and Soc. Issu., Concep. Strate. Plant Sci., pp. 195–243.

Rocha da, I. M.; V. A. Vitorello; J. S. Silva; S. L. Ferreira-Silva; R. A. Viegas; E. N. Silva and J. A. Silveira (2012). Exogenous ornithine is an effective precursor, and the delta-ornithine amino transferase pathway contributes to proline accumulation under high N recycling in salt-stressed cashew leaves. J. Plant Physiol., 169: 41–49.

Rodrigues, C. R. F.; E. N. Silva; R. da M. Moura; D. C. dos Anjos; F. F. F. Hernandez, R. A. Viégas (2014). Physiological adjustment to salt stress in R. communis seedlings is associated with a probable mechanism of osmotic adjustment and a reduction in water lost by transpiration. Ind. Crops Prod., 54: 233–239.

Ronceret, A. and P. Bolaños-Villegas (2024). Plant reproduction research in Latin America: Toward sustainable agriculture in a changing environment. Plant-environ. Inter. (Hoboken, N. J.), 5 (3), 10143.

Snedecor, G. W. and W. G. Cochran (1980). "Statistical Methods". 7th Edition, Iowa State Univ. Press, Ames, USA.

Sonneveld, E.; C. E. van den Brink; L. G. Tertoolen; B. van der Burg and P. T. van der Saag (1999). Retinoic acid hydroxylase (CYP26) is a key enzyme in neuronal differentiation of embryonal carcinoma cells. Dev Biol., 213 (2): 390-404.

Spormann, S.; P. Nadais; F. Sousa; M. Pinto; M. Martins; B. Sousa; F. Fidalgo and C. Soares (2023). Accumulation of Proline in Plants under Contaminated Soils—Are We on the Same Page? Antioxidants., 12 (3): 666.

Stroganov, B. P. (1964). Physiological Basis of the Salt Tolerance of Plants (under Different Types of Soil Salinization). Izd-vo nauk Akadem. SSSR Sci., Moscow, 546.

Tiwari, J. K.; A. D. Munshi; R. Kumar; R. N. Pandey; A. Arora; J. S. Bhat and A. K. Sureja (2010). Effect of Salt Stress on Cucumber: Na+/K+ Ratio, Osmolyte Concentration, Phenols and Chlorophyll Content. Acta Physio. Plant., 32: 103-114.

Torre-Ruiz, De La N.; V. M. Ruíz-Valdiviezo; C. I. Rincón-Molina.; M. A. Rodríguez-Mendiola.; C. Arias-Castro; F. A. Gutiérrez-Miceli; H. Palomeque-Domínguez and R. Rincón-Rosales (2016). Effect of plant growth-promoting bacteria on the growth and fructan production of Agave americana L. Brazil. J. Micro., 78 (47): (1-10) 587 – 596.

Walter, G. R. and E. Nawacki (1978). Alkaloid biology and metabolism in plants. Planum Press, N. Y., p. 152.

Weatherly, P. E. (1962). Examination of the moisture stress on carbohydrate development and growth in plants. Amer. J. Bot., 41: 316-320.

Woodrow, P.; L. F. Ciarmiello; M. G. Annunziata; S. Pacifico; F. Iannuzzi; A. Mirto; L. D’Amelia; E. Dell’Aversana; S. Piccolella; A. Fuggi and C. P. Durum (2017). Wheat seedling responses to simultaneous high light and salinity involve a fine reconfiguration of amino acids and carbohydrate metabolism. Physiol. Plant, 159: 290–312.

Yamada, M.; H. Morishita; K. Urano; N. Shiozaki; K. Yamaguchi-Shinozaki and K. Shinozaki (2005). Effects of free proline accumulation in petunias under drought stress. J. Exp. Bot., 56 (417): 1975-1981.