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Changes in Contents of Free Amino Acids in Germinating Seed Organs of Three *Senna* Species under Temperature, Osmotic Stress and Zinc Concentration

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Abstract: The present study deals with the effects of osmotic stress, Ψ_s, temperature (T) and zinc (Zn) concentrations (in the physiological range) on the germination processes of seeds in three plant species of different ecological affiliation, namely: *Senna alexandrina*, *Senna italic* (native to hot deserts) and *Senna occidentalis* (a wild mesophytic plant). Total content, specific and allocation of soluble and potentially free amino acids in the embryonic axis (radicle and hypocotyl) were determined. It was observed that, addition of zinc to the seed incubation medium improved the adjustment of radicles to water deficiency conditions through increasing the allocation of free amino acids into the radicles and hence increasing the osmotic potentials of radicles. Also, zinc induced additional adaptation of the plants to extreme temperatures through increasing total osmotically active amino acids in the radicle. While, glutamic was the dominant amino acid in radicles of *S. alexandrina* and *S. italica* (xerophytes), proline was the dominant in *S. occidentalis* (mesophyte). The statistical analysis indicated that the trifactorial interaction (ΨsxTxZn) had the major effect on the total free amino acids of the three plants.

Keywords: osmotic stress, temperature, zinc, *Senna* species, free amino acids, trifactorial interaction.

1 Introduction

When plants experience environmental stresses such as drought, salinity and temperature, they activate various metabolic and defense systems to survive [8,17]. The adaptability of plant species to high salt concentrations in the soil by lowering tissue osmotic water potential is accomplished by accumulation of osmotic solutes such as soluble carbohydrates, proteins and free amino acids [7]. Also, zinc is a heavy metal can have detrimental effects on many vital processes in plant cells. Therefore, zinc as element is an essential for plant growth, development, and many metabolic processes. Amino acids, as precursors for protein formation are necessary for growth in the seedling stage. As a part of metabolic osmolytes, it plays a partial role in osmotic adjustment of seedling organs especially the radical. In this respect, some researchers refer to specific amino acids (e.g. proline, and glycine betain) as osmo-protectants under drought stress conditions due to their increase under drought stress [1,15,10]. The aim of this present work is to study the effect of different levels of osmotic water stress, temperature and zinc concentration in the germination medium on amino acid content of germinating seed organs of 3 *Senna* species of different ecological affiliations. More important is to evaluate the interactive effects of such factors on the content of amino acids in the developing organs of the germinating seeds.

2 Materials and Methods

Three *Senna* species belonging to ecologically different habitats were investigated. These are:*Sennaalexandrina* Mill (*Cassia senna*). *Sennaitalica* Mill. (*Cassia italica*) and *Senna occidentalis* (L.) Link. Preliminary germination tests performed before experimentation indicated a high germination percentage, reaching about 100% in these seeds; *Senna italica* seeds have naturally hard coats which therefore cause their dormancy. To induce germination in such case, special pretreatments were required, without which these seeds failed to attain the proper germination percentage. The mechanical scarification, by scratching the seed coats with the aid of abrasives, was found sufficient to

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ensure 100% germination.

2.1 Adjustments of Incubation Temperatures

Incubators with air circulation which allow control of temperature between 15°C and 35°C were used in testing temperature effect on germination. The incubators were kept constantly dark during the period of incubation. The tests were run at: 15°C, 20°C, 25°C, 30°C and 35°C.

2.2 Adjustments of Simulated Reduced Osmotic Water Potential, Ψs and Zinc Concentration (Zn)

The effect of decreased osmotic water potential on germination was simulated by using sodium chloride + calcium chloride solutions as substrate media for germinating seeds. Solutions having different water potentials, ψ_s, were prepared by dissolving certain amounts of sodium chloride (NaCl + calcium chloride ($CaCl_2$) in water, according to preconstructed calibration curves, keeping the sodium adsorption ratio (SAR) constant (1/8). Solutions having different water potentials with zinc (ψ_s+ Zn), were prepared by dissolving certain amounts of NaCl + $CaCl_2$ in zinc solution. The treatment solutions prepared thus are of certain levels of treatment combinations. Seeds were exposed to the following range of osmotic potentials: 0, -0.3, -0.7, -1.0, -1.5 MPa. For each species, another series of zinc solutions (0, 2, 5 and 8 ppm) at the same different levels of osmotic water potential. (ψ_s+ Zn) were prepared. The highest stress level for each seed kind represents the maximum tolerance limit (Least germination) as revealed by preliminary tests. Sets of 4 Petri-dishes were randomly assigned to each osmotic potential (ψ_s) level with or without zinc, then incubated at the specific temperatures as explained before. In order to have the data comparative under different conditions, incubation was terminated after 15 days of sowing, a period long enough to cover any delay of germination due to stress especially at low water potential levels or extreme temperature treatments.

For extraction, the radicle, hypocotyl and storage tissue were excised, washed rapidly in distilled water and rapidly dried between filter paper layers. The excised organs were then weighed, immediately crushed and extracted in 10 ml of ice-cold distilled water [3]. The extracts were kept in deep freeze until the time of analysis for total free amino acids.

2.3 Determination of Total Free Amino Acids

a-Total free amino acids were determined according to procedures described by [11].

b- Separation and identification of some specific water soluble amino acids: -

Nine water soluble amino acids have been separated and identified, then estimated qualitatively and quantitatively using Agilent Capillary electrophoresis apparatus Model G 1600 Ax Germany, available at the Analytical Chemistry Unit (ACAL) of Assiut University. These amino acids include: glutamic acid, tyrosine, glycine, alanine, serine, threonine, proline, valine and phenyl alanine.

2.4 Statistical Analysis:

The significance of the effects of single factors and their interactions were determined by analysis of variance. Based on the significance status, the magnitudes of the relative effect of each single factor and its interaction was determined by using the coefficient of determination (importance value, η^2), which is considered a test used to indicate the degree of control of each factor and its interaction on the total free amino acids [13,14], as applied by [2].

3 Results and Discussion

3.1 Total Free Amino Acids

Proteins involved in carbohydrate, energy and amino acid metabolism constituted about ¼ of total proteins extracted in the germinating seeds [5]. The induction of germination by rehydration of the seeds leads to an increase in respiration and metabolic activity that allows carbon and nitrogen reserves to be mobilized [12]. These metabolic changes were characterized by an increase in total amino acids (AA), particularly glycine and ammonium [9]. The investigated species exhibited variability in total free amino acids (AA) content at different treatments. In general, AA content was higher in *S. occidentalis* than that in the other two species. The increased free AA was shown at moderate temperatures with low and high amended zinc concentrations.

In *S. alexandrina*, (Figure 1) the total amino acids contents of germinating seeds responded readily to temperature changes. At Zn 5 ppm, maximum content of amino acids (211.0 mg.g^{-1} d. wt.) occurred at 35ºC under -0.7 Mpa. At 30ºC, AA content reached a peak at a higher level of osmotic water potential (-0.3 MPa) and zinc concentration (8 ppm). At the lowest osmotic water potential level (-1.5 MPa), there is an increase in amino acids content in absence or presence of low zinc concentration and at temperature 25ºC.

At relatively high temperatures, it is found that free amino acids allocation to the embryonic axis gradually increased with increasing water stress. While, at the lowest temperature (15ºC), the cotyledon gained a larger proportion of amino acids than the radicle and hypocotyl with highest Zn concentration (8 ppm) and osmotic water potential (-0.3 MPa). The same is true at 35ºC but in absence of zinc (Figure 2).

In *S. italica*, in the absence of zinc with low osmotic water potential (-1.5 MPa), there was an increase in amino acids content at 20 and 25ºC. Amino acids content reached a high value (277.9 mg.g^{-1} dry wt.) at 25ºC under moderate osmotic water potential (-0.7 MPa). In general, the same temperature yielded a maximum AA content (358.4 mg.g^{-1} dry weights) in seedling at different Zn levels especially at high water stress. On the other hand, high zinc concentration (8 ppm) showed a remarkable AA peak (142.5 mg.g^{-1} dry weight) at low temperature (15ºC) in absence of water stress (Figure 3).

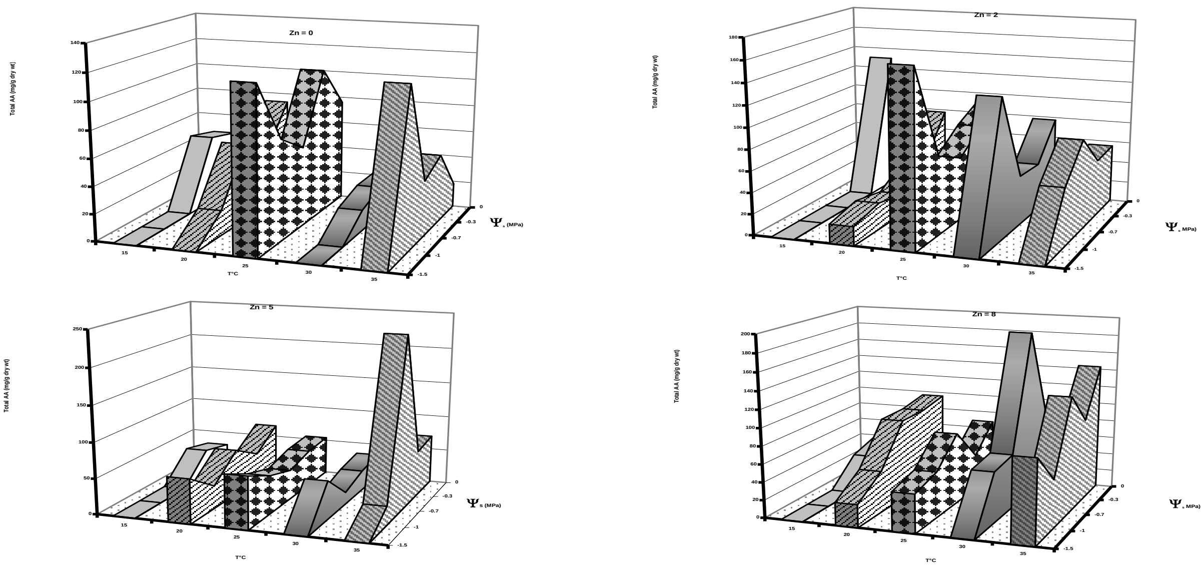
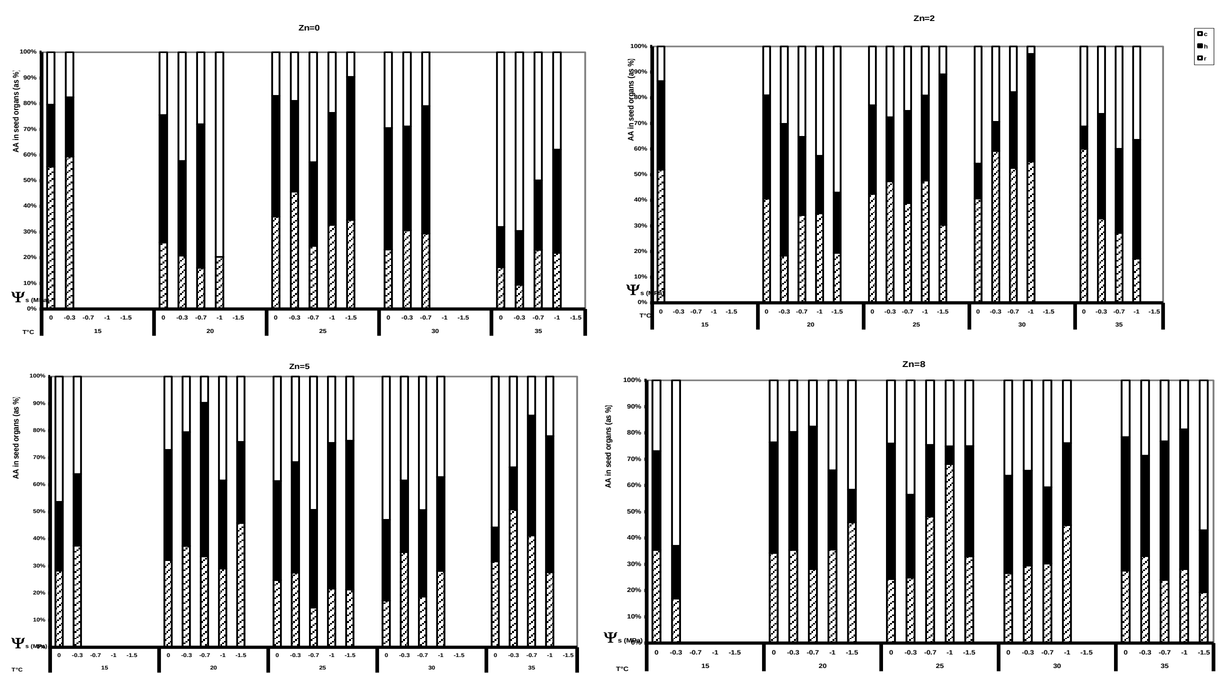


Figure 1. Total content of amino acids (AA) in germinating *Senna alexandrina* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.



r = radicle h = hypocotyl c = storage tissue

Figure 2. Relative distribution (as %) of amino acid (AA) in germinating *Senna alexandrina* seed organs at different osmoticwater potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

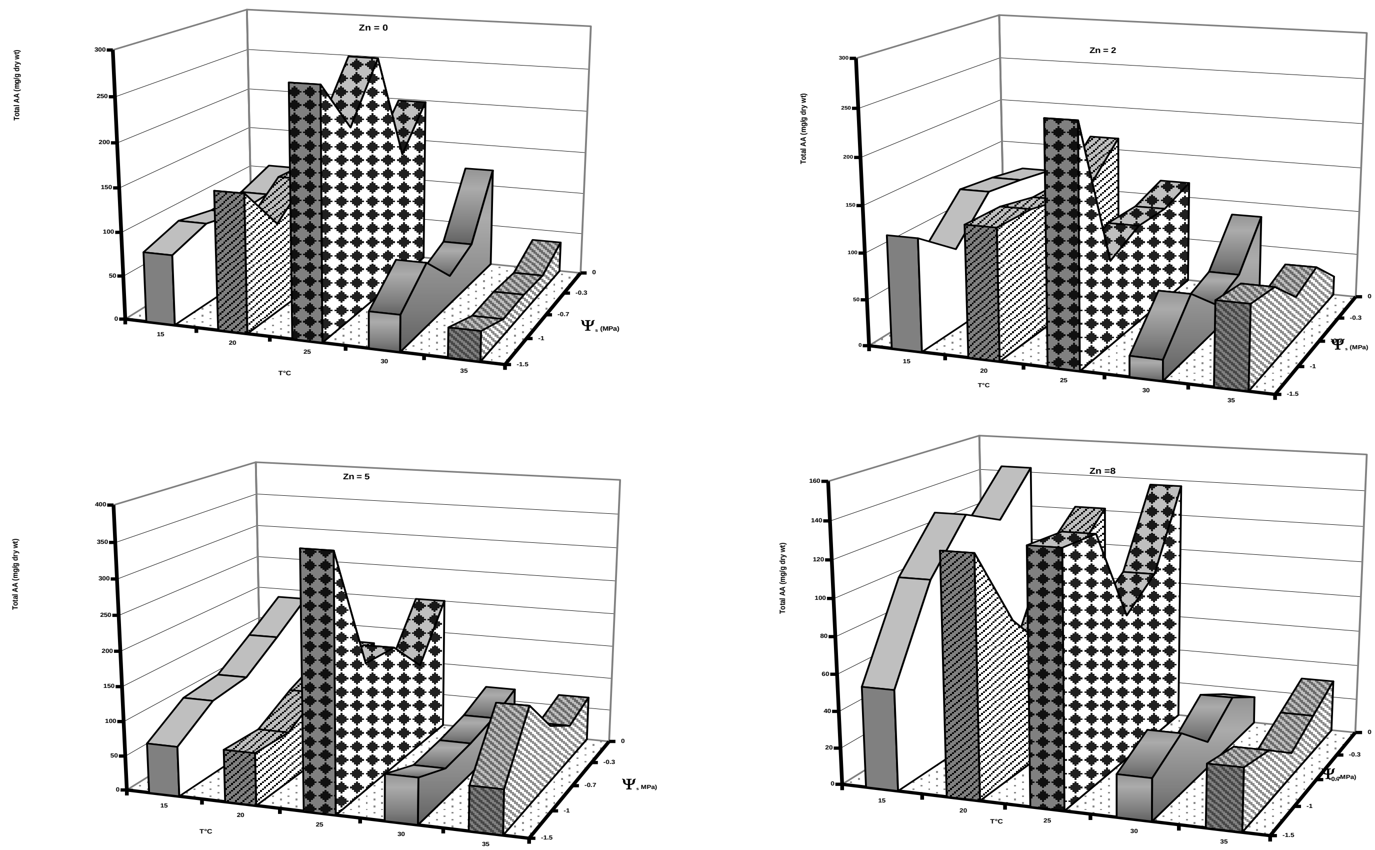


Figure 3. Total content of amino acids (AA) in germinating *Senna italica* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

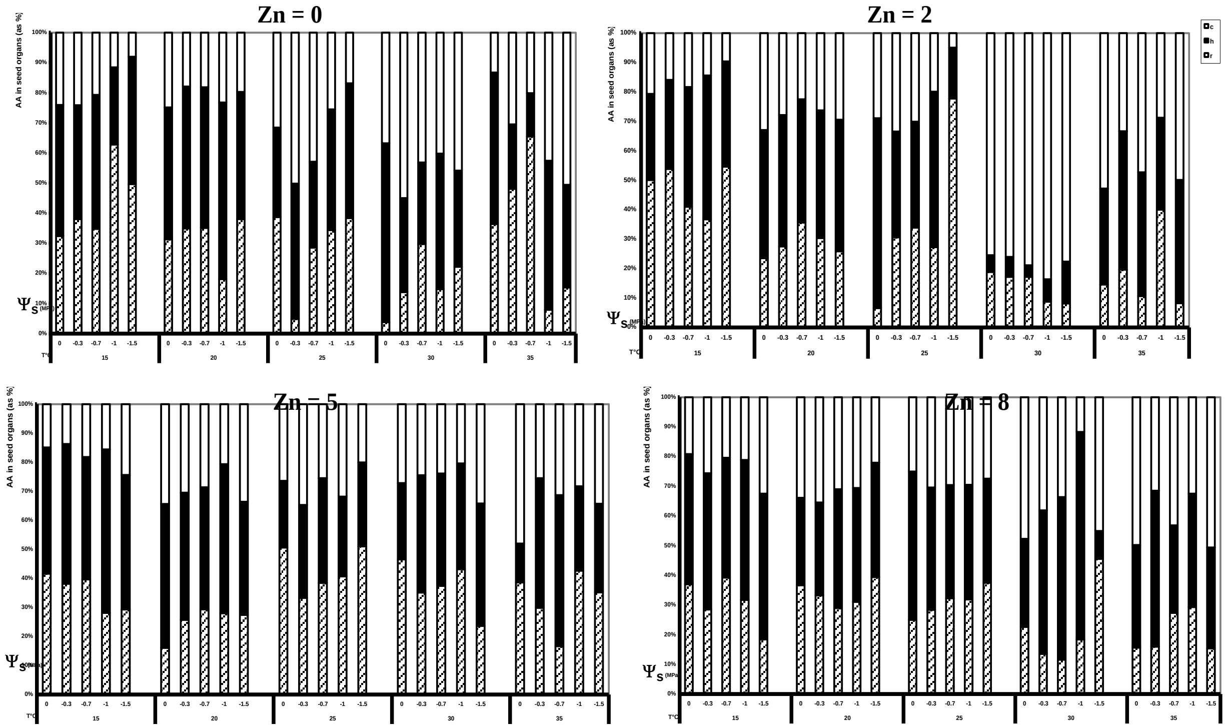
Both in absence or presence of zinc, the embryonic axis gained a larger share of amino acids than the storage tissue at all temperatures with gradual decrease in osmotic water potential. At temperature 30ºC, there was an exception where low Zn concentration (2 ppm) induced increase in amino acid content in the storage tissue rather than in the embryonic axis at all osmotic water potential levels (Figure 4).

In our work, total AA content were higher in *S. occidentalis* than that in the other two species. The increased total free AA was shown at moderate temperatures with low and high amended zinc.Total amino acids content in experimented seeds mostly decreased with the decrease in osmotic water potential.

For *S. occidentalis*, (Figure 5), the increase in amino acids content was observed in the absence of zinc at all experimental temperatures with osmotic potentials (0 to -0.7 MPa). In general, it is observed that maximum contents of amino acids in this species were observed at optimum and high temperatures. Low zinc concentration at optimum temperature 25ºC induced increase in amino acids content with osmotic water potential (-0.3 MPa). Moderate zinc concentration showed two peaks at 25 & 35ºC under -0.7 & 0 MPa, respectively. The highest value (420.1 $mg.g^{-1}$ dry weight) was detected at high zinc and temperature levels under -1 MPa.The free amino acids content was higher in the embryonic axis (hypocotyl has the largest proportion) than in storage tissue whether in absence or presence of zinc (Figure 6). At extreme temperature 35ºC and low zinc concentration, the storage tissue had the highest proportion of amino acids in absence of water stress (0 MPa).

Plants responded to temperature change through increasing amino acid levels in the whole germinating seeds especially under low osmotic water potentials (at moderate and high zinc concentrations). This increase was clearly noted in *S. alexandrina* and *S. occidentalis* at high temperature (35ºC) and in *S. italica* at optimum temperature (25ºC). On the other hand, Nein and Vyas [18] reported that, the levels of free amino acids were not much affected by high temperature in both genotypes of cluster bean seedlings they have investigated.

Information concerning how an embryo mobilizes its internal reserves during the early stage of germination can provide insights into the metabolic process of germination [6]. The data hitherto presented, indicated that, translocation of amino acids to the embryonic axis of the three plants was greatly noticed at all temperatures, osmotic water potentials and zinc concentrations (except at 35ºC in the absence of zinc, in unstressed and -0.3 MPa in *S. alexandrina*, as well as, at 30ºC with low zinc concentration over all osmotic water potentials in *S. italica* where storage tissue had a large share of amino acids).This means, probably, that there is a problem in translocation to the embryonic axis.



r = radicle h = hypocotyl c = storage tissue

Figure 4. Relative distribution (as %) of amino acid (AA) in germinating *Senna italica* seed organs at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

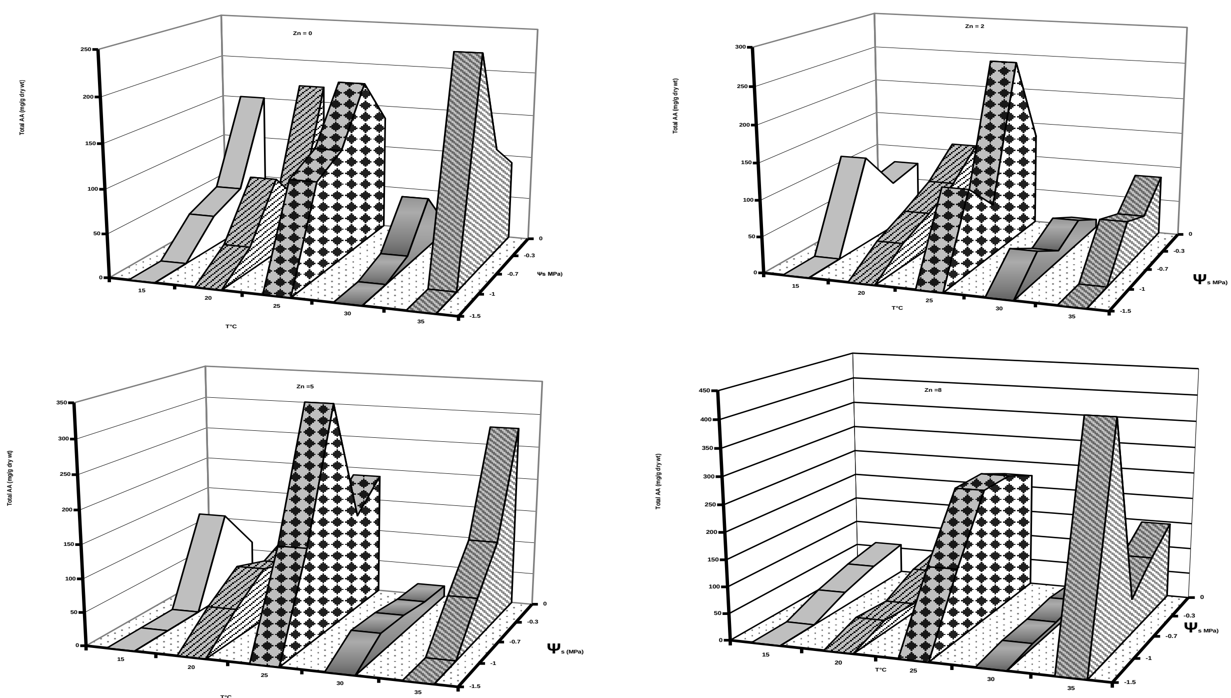
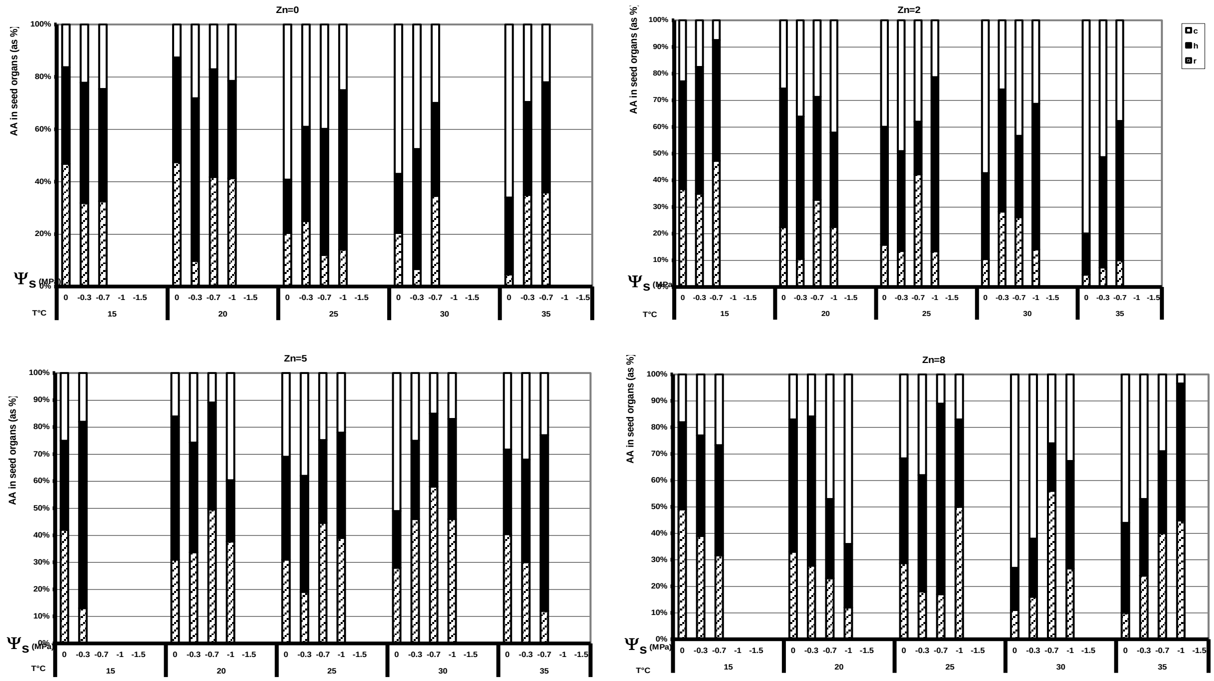


Figure 5. Total content of free amino acids (AA) in germinating *Senna occidentalis* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.



r = radicle h = hypocotyl c = storage tissue

Figure 6. Relative distribution (as %) of amino acids (AA) in germinating *Senna occidentalis* seed organs at different osmotic potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

Table 1.F& η^2 values for the effect of temperature (T), zinc (Zn), osmotic water potential (□$_s$) and their interactions on total free amino acids of seedling organs in investigated species

Species		*S. alexandrina*						*S. italica*						*S. occidentalis*					
		Radicle		Hypocotyl		cotyledon		Radicle		Hypocotyl		cotyledon		Radicle		Hypocotyl		cotyledon	
Source of variance	DF	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2	F	η^2
□$_s$	4	36.94**	0.06	34.32**	0.04	39.85**	0.13	22.59**	0.04	5.32**	0.01	10.87**	0.03	152.17**	0.12	291.68**	0.16	193.16**	0.28
T	4	57.67**	0.07	107.47**	0.13	78.16**	0.26	202.17**	0.39	201.62**	0.55	153.62**	0.36	152.64**	0.12	424.40**	0.23	226.55**	0.33
Zn	3	26.46**	0.03	31.85**	0.03	27.25**	0.07	30.71**	0.04	24.66**	0.05	24.29**	0.04	51.03**	0.03	27.03**	0.01	0.78	0.009
□$_s$×T	16	30.66**	0.18	51.90**	0.03	8.03**	0.11	30.22**	0.23	6.14**	0.07	8.37**	0.08	23.86**	0.08	52.91**	0.11	41.89**	0.25
□$_s$×Zn	12	24.04**	0.11	31.73**	0.11	6.42**	0.06	5.61**	0.03	1.68	0.01	3.50**	0.02	40.66**	0.10	25.89**	0.04	3.34**	0.02
T×Zn	12	41.76**	0.18	29.62**	0.11	9.14**	0.09	13.95**	0.08	17.63**	0.14	42.01**	0.29	40.88**	0.10	46.56**	0.08	1.89*	0.008
□$_s$×T×Zn	48	19.48**	0.35	23.53**	0.34	7.39**	0.29	7.58**	0.18	4.80**	0.16	6.35**	0.18	47.01**	0.45	58.09**	0.37	6.35**	0.11

* Significant at $P< 0.05$ ** Significant at $P< 0.01$

In this respect, it was found that the interaction (Ψ_s x T x Zn) has the predominant effect on amino acids content in the organs of *S. alexandrina* and *S. occidentalis* except for storage tissue in *S. occidentalis* where temperature can be considered the major factor(Table1). This suggests that the rate of AA translocation from storage tissue to the embryonic axis was controlled by the trifactorial interaction. In *S. italica*, temperature has the predominant effect in the three organs. This is in agreement with the data recoded by El Sharkawi andFarghali [4].

3.2 Specific Water Soluble Amino Acids

Water soluble amino acids were identified and quantitatively estimated in selected treatment combinations (Table 2, 3 &4). These included glutamic acid, tyrosine, glycine, alanine, serine, threonine, proline, valine and phenyl alanine.

In *S. alexandrina*, at zero water stress (0 MPa), high content (compared to other treatments) of amino acids namely: glycine (Maximum value= 0.233 $mg.g^{-1}$ d. wt.), alanine, serine, threonine, proline and valine were found at low temperature (15ºC) in absence of zinc, while at osmotic water potential of -0.3 MPa the maximum concentration of glutamic acid was present at the same low temperature and zinc concentrations. At 30^{0}C, phenylalanine content (0.202 $mg.g^{-1}$ dry wt.) was present mostly in the absence of zinc at -0.7 MPa. (Table 2).

In *S. italica*, at low temperature 15ºC, tyrosine, glycine, alanine, proline and valine were mostly present in the absence of zinc and at high osmotic water potential. Whereas, at moderate zinc concentration (5 ppm) serine and phenylalanine were dominant at the same low temperature in absence of water stress. At temperature 25ºC, glutamic acid was mostly abundant in the absence of zinc at osmotic water potential -1MPa, while a high content of threonine was observed at 30ºC and moderate zinc concentration. (Table 3).

In *S. occidentalis*, moderate zinc concentration (5 ppm) yielded a maximum content of glycine, serine, threonine and valine at temperature 30ºC with osmotic water potential -1 MPa. Meanwhile, in absence of zinc, proline was present with considerable high content (0.818 $mg.g^{-1}$ d. wt.) at low temperature 15ºC and at high osmotic water potential, alanine was highly present at zinc concentration (5 ppm). The remaining investigated amino acids were dominantly observed at temperature 25ºC with osmotic water potential -0.7 MPa and in the absence of zinc (Table 4).

Table 2. Specific water soluble amino acids content ($mg.g^{-1}$ dry wt.) at different treatments of temperature (T), zinc (Zn) and osmotic water potential (□$_s$). in the radicles of *S. alexandrina* germinating seeds.

Treatments / Amino Acids	T_{15},Ψ_0,Zn_0	$T_{15},\Psi_{-0.3}, Zn_0$	$T_{30},\Psi_{-0.7}, Zn_0$	$T_{30},\Psi_{-0.7}, Zn_5$
Glutamic acid	0.009	0.085	0.005	0.002
Tyrosine	0.197	-------	0.202	0.040
Glycine	0.233	-------	0.006	0.131
Alanine	0.055	-------	------	0.006
Serine	0.057	-------	------	------
Threonine	0.017	-------	0.009	0.006
Proline	0.219	-------	0.177	0.007
Valine	0.231	-------	0.202	0.007
Phenyl Alanine	0.036	-------	0.202	0.012

Table 3. Specific water soluble amino acids content ($mg.g^{-1}$ d. wt.) at different treatments of temperature (T), zinc (Zn) and osmotic water potential (□$_s$) in the radicles of *S. italica* germinating seeds.

Treatments / Amino Acids	T_{15}, Ψ_0, Zn_0	T_{15}, Ψ_0, Zn_5	$T_{25}, \Psi_{-1.0}. Zn_0$	T_{30}, Ψ_{-1}, Zn_5
Glutamic acid	0.007	0.019	0.103	0.031
Tyrosine	0.143	0.130	-------	0.060
Glycine	0.169	------	-------	-------
Alanine	0.040	0.019	-------	0.015
Serine	0.041	0.072	-------	0.011
Threonine	0.012	0.020	-------	0.028
Proline	0.159	0.022	-------	0.028
Valine	0.167	0.016	-------	0.001
Ph.Alanine	0.026	0.038	-------	0.001

Table 4. Specific water soluble amino acids content (mg.g^{-1} d. wt.) at different treatments of temperature (T), zinc (Zn) and osmotic water potential (□$_s$). in the radicles of *S. occidentalis* germinating seeds

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Treatments / Amino Acids	T_{15},Ψ_0, Zn_0	T_{15},Ψ_0, Zn_5	$T_{25},\Psi_{-0.7}, Zn_0$	$T_{30},\Psi_{-1.0}, Zn_5$
Glutamic acid	0.019	0.045	0.179	0.007
Tyrosine	-----	-----	0.119	-----
Glycine	-----	0.030	0.013	0.371
Alanine	0.032	0.055	0.050	0.053
Serine	0.378	0.005	0.034	0.423
Threonine	0.015	0.005	0.016	0.275
Proline	0.818	-----	0.006	-----
Valine	0.041	0.006	0.011	0.275
Phenyl Alanine	-----	-----	0.199	-----

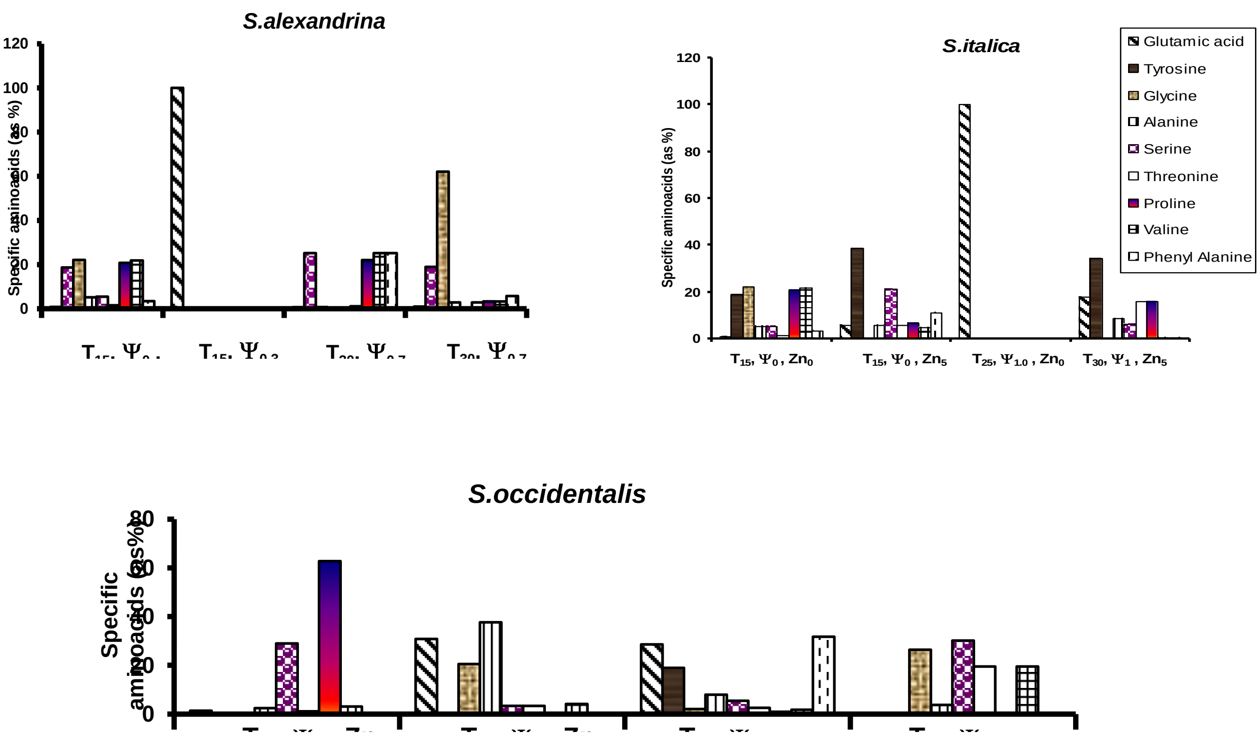

Figure 7. Relative content of different amino acids expressed as % of total content showing relative dominance of each among other A.A. in each plant.

The capillary electrophoresis indicated that proline was the predominant amino acid in the radicles of *S. occidentalis* and has the maximum levels over all amino acids in the radicles of the other two species, while, glutamic acid was the dominant in the radicles of *S. alexandrina* and *S. italica* with high concentrations. These high concentrations of proline and glutamic acid were present at low temperature in the absence of both water stress and zinc. On the contrary, it was shown that the decrease in germination was accompanied by an increase in proline of embryos after NaCl treatments [16].

4 Conclusion

Apparently, addition of zinc to the seed incubation medium improved the adjustment of radicles to water deficiency conditions through increasing the allocation of free amino acids into the radicles and hence increasing the osmotic potentials of radicles. Also, zinc induced additional adaptation of the plants to extreme temperatures in the radicle. While, glutamic was the dominant amino cid in radicles of *S. alexandrina* and *S. italica* (xerophytes), proline was the dominant in *S. occidentalis* (mesophyte). The statistical analysis indicated that the trifactorial interaction (ΨsxTxZn) had the major effect on the total free amino acids of the three plants.

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