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Zn Induced Soluble Proteins in Germinating *Senna* Seeds under Temperature and Osmotic Water Potential

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Abstract: The present study aims to investigate the effects of osmotic water potential simulated by reduced osmotic water potential, Ψ_s, temperature (T) and zinc (Zn) addition to the seed incubation medium and to evaluate the effects of their interactions on soluble proteins in germinating seeds of three plant species of different ecological affiliation. The experimented species were namely: *Senna alexandrina*, *Senna italic* (native to hot deserts) and *Senna occidentalis* (a wild mesophytic plant). It was observed that, the presence of high Zn concentration yielded a high content of soluble proteins under low osmotic water potential and high temperature. Also, addition of zinc improved the adjustment of radicles to water deficiency conditions through increasing the allocation of water soluble proteins into the radicles. The germinating seeds in *S. occidentalis* (wild mesophyte) was characterized by the presence of high molecular weight protein bands, whereas radicles of xerophytes contain low molecular weight protein bands. The statistical analysis indicated that the trifactorial interaction (ΨsxTxZn) had the major effect on the soluble proteins in different organs of the three-plant species. Exceptionally, the effect of osmotic water potential was the dominant in storage tissue proteins of *S. occidentalis*.

Key words: Multi-factorial interaction -Soluble proteins – Zinc – Temperature-Osmotic water potential – *Senna sp*.

1 Introduction

Salinity and temperature may control seed germination due not only to the intrinsic characteristics of each species, but also to the differential effects of temperature on the osmotic and ionic components of salinity, on one side and the chemical nature of the ions involved on the other. In general, the data revealed by multi-factorial interaction indicated that the prominent factors characteristic of desert do not affect the germination process separately (El Sharkawi*et al.*1999). Thus, such interactions may change from synergistic to antagonistic or vice-versa [14,22]

In seeds, there are two distinct metabolic centers:1- the storage organs where reserve metabolites are hydrolyzed and,2- the elongating embryo axis where incoming nutrients are utilized for the synthetic activities associated with cell expansion [12]. Imposed water stress reduces the ability of seeds to germinate due to inhibition of water uptake and the activity of enzymes [8]. Proteins involved in carbohydrate energy and amino acid metabolism constituted about ¼ of total proteins extracted in the germinating seeds [5]. Moreover, 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 [18]. Hence, Information concerning how an embryo mobilizes its internal reserves during the early stage of germination can provide insights into the metabolic process of germination [7]. In addition, deficiency in certain ions especially those related to metabolic activities (such as Zn) of desert soils in general, have to be accounted for. Therefore, zinc is an activator of several enzymes, plays an important role in DNA and RNA metabolism and Zn encouraged protein synthesis [16,23].

The aim of the present work is: 1-To run a comparative study between soluble proteins (low molecular weight) in the three-species belonging to the same genus (*Senna*) of different ecological affiliations.2- Evaluation of the significance of the effect of each ecological factor (salinity, temperature and Zn supply) tested. 3-Concerning the effect of reduced water potential, the osmotic water potential was chosen for this study since the three-species concerned are differently sensitive to salinity. 4- With respect to nutrient deficiency, zinc was chosen as a micro nutrient since desert soils are deficient in zinc and such micro nutrient is involved in the enzymology of metabolites.

2 Materials and Methods

In this experimental work seeds of three *Senna* species belonging to ecologically different habitats were tested. These are:*Senna alexandrina* Mill (*Cassia senna*).

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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 ensure nearly 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 soluble metabolites.

2.3 Determination of water soluble proteins

a-Total soluble proteins were determined according to [15].
b- Proteins were analyzed by acrylamide gel electrophoresis (SDS-PAGE, according to Laemmli (1970) in the first dimension available at Department of Genetics, Faculty of Agriculture, Assiut University and the Gel-Pro Analyzer package (Media Cybernetica 1993-97) was used in determination of the molecular weight and percentage of water soluble proteins.

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 tested parameters [19,20], as applied by [2].

3 Results and Discussion

3.1 *Total and relative distribution of water soluble proteins*

Zn is most probably used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses [1]. Generally, soluble proteins content of experimented seeds decreased with the reduction in osmotic water potential. In some cases, zinc concentrations enhance soluble proteins induction especially at low osmotic water potential. According to Nein and Vyas [17] increased temperature promotes the activities of nitrate reductase, nitrite reductase, glutamate dehydrogenase and protease (proteinase). Obviously, an increase in soluble proteins content of S. alexandrina (581.6 mg.g-1 dry weight) was found at low osmotic water potential (-1.5 MPa) with optimum temperature 25ºC. The similar increase occurred at high temperature (35ºC) regardless of zinc concentration (Figure 1). As shown in figure (2), in the absence of zinc at all stress levels and temperatures; accumulation of soluble proteins from storage tissue to embryonic axis (especially to radicle) occurred. It's quite clear, that zinc concentration (2 ppm) induced increases in soluble proteins of radicle at a wide range of temperature (25-35ºC). At low osmotic water potential level (-1.5 MPa) storage tissue had more soluble proteins than the embryonic axis at all temperatures with the range 5-8 ppm of Zn concentrations.

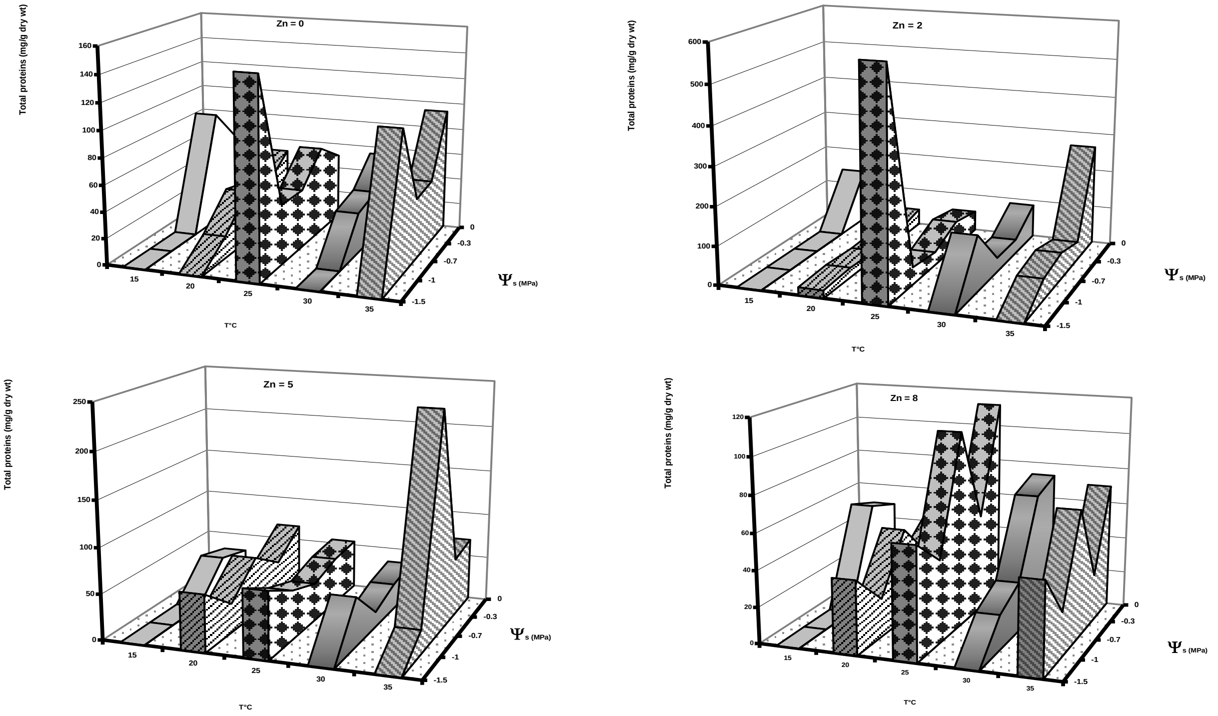


Figure (1): Total content of soluble proteins in germinating *Sennaalexandrina* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

In *S. italica*, (Figure 3), it was found that at low temperature (15°C) and zinc concentrations (0, 5 and 8 ppm), an increase in soluble proteins content which reached its maximum of 125.7 mg.g^{-1} dry wt. at moderate osmotic water potentials. A remarkable peak was observed at 0 MPa at 35°C in the absence of zinc which shifted to 30°C at low zinc concentration (2 ppm). Regardless of osmotic water potentials, total proteins decreased gradually with the increase in temperature at Zn concentrations ranging from 5 to 8 ppm.

Soluble proteins showed relatively the same pattern of response to temperature in absence and in presence of zinc. As shown in figure (4), under water stress, the embryonic axis gained a larger proportion of soluble proteins than the storage tissue (a relatively larger share of soluble proteins is found in the radicle), especially at low temperatures (15 and 20°C). At temperature 30°C, the absence of Zn delayed the formation of soluble proteins to the embryonic axis with low osmotic water potential level (-1.5 MPa). This is true at the highest experimental temperature (35°C) at Zn concentration 5 pp m.

In *Senna occidentalis*, (Figure 5), it was found that in the absence of zinc, a high soluble proteins content was present at 30°C under high osmotic water potential. An increase in soluble proteins (295.8 mg.g-1 dry wt.) was usually observed at moderate Zn concentration (5 ppm) with increase in temperature. Low Zn concentration (2 ppm) caused a gradual increase in soluble proteins with the decrease in water stress at a wide range of temperatures. The presence of high Zn concentration yielded a high content of soluble proteins under low osmotic water potential and high temperature (35°C).

High content of proteins in the embryonic axis at temperature 30°C in Zn- untreated and low treated (2 ppm) seedlings was observed, and less content was observed at 35°C under both Zn levels. At zinc concentrations of 5 and 8 ppm, protein content increased at 35°C, especially at moderate stress level (Figure 6). In general, soluble proteins were conserved in storage tissue of *S. occidentalis* more than in the other two species, particularly at high temperature and low Zn concentration (2 ppm).

During germination, enzymatic hydrolysis of storage proteins in the endosperm forms a reservoir of low molecular weight peptides and amino acids, which are translocated to supply organic nitrogen to the growing seedling and protein levels were slightly modified during seed germination [13]. In the investigated three experimental plants, increased amount of soluble proteins was found in the radicle except for *S. occidentalis* where the storage tissue had higher amounts of soluble proteins at extreme high temperature (35°C) at all osmotic water potentials, particularly with low zinc concentrations. This means that, the adaptation of these plants to heat stress induced accumulation of water binding molecules and compatible solutes which related to enhance thermostability [5]. All single factors and their interactions show significant effect on soluble proteins of radicle, hypocotyl and storage tissue (With some exceptions). Values of the determination coefficient (η2) which indicate the relative role played by each single factor (Ψs, T and Zn) and their interactions (Ψs x T, Ψs x Zn, T x Zn and Ψs x T x Zn) confirmed that, Ψs x T x Zn interaction has the dominant effect on soluble proteins in different organs of the three *Senna* species. Exceptionally, the effect of osmotic water potential was the dominant in storage tissue proteins of *S. occidentalis* (Table 1).

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
□	4	10.08**	0.02	13.51**	0.06	68.15**	0.21	16.56**	0.12	6.91**	0.03	4.10**	0.04	32.23**	0.11	69.78**	0.15	204.06**	0.55
T	4	35.11**	0.06	18.28**	0.09	67.52**	0.21	20.96**	0.15	28.81**	0.11	3.50**	0.04	15.93**	0.06	16.84**	0.04	40.44**	0.11
Zn	3	40.77**	0.06	1.41	0.01	19.81**	0.05	3.73*	0.02	7.21**	0.02	0.77	0.01	9.13**	0.02	11.58**	0.02	2.73*	0.01
$□_s$×T	16	28.95**	0.21	17.64**	0.33	13.30**	0.17	3.66**	0.11	12.73**	0.19	5.45**	0.23	9.47**	0.13	15.68	0.14	12.52**	0.13
$□_s$×Zn	12	16.23**	0.09	6.34**	0.09	5.70**	0.05	3.18**	0.07	7.55**	0.08	2.31**	0.07	7.83**	0.08	10.39**	0.07	2.02*	0.02
T×Zn	12	16.12**	0.09	4.72**	0.07	7.04**	0.07	10.43**	0.23	12.93**	0.14	6.17**	0.19	17.34**	0.18	13.20**	0.09	4.33**	0.04
$□_s$×T×Zn	48	21.36**	0.47	6.44**	0.36	6.67**	0.25	3.37**	0.30	9.56**	0.43	3.30**	0.42	9.75**	0.41	18.91**	0.50	4.91**	0.16

Table (1): F& η^2 values for the effect of temperature (T), zinc (Zn), osmotic water potential ($□_s$) and their interactions on soluble proteins of seedling organs in ivestigated species.

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

3.2 Electrophoresis analysis of soluble proteins (of radicles)

The SDS-PAGE analysis of water soluble protein fractions revealed differences in the banding profile patterns, represented by their presence or absence and their intensity, in radicles of the three chosen experimental species with specific treatments as shown in photo-plates (Photo-plates A, B & C).

In *S. alexandrina*, low and moderate molecular weight bands of proteins (9.1 – 118.9KD) were observed at all temperatures, zinc treatments and osmotic water potential levels. At temperature 30ºC, only moderate molecular weight protein bands were detected with low osmotic water potential (-1MPa) and zinc concentrations 5 and 8 ppm (Table 2).

In *S. italica* (Table 3), protein bands were found with molecular weights ranging from 22.6 -114.0 KD at low temperature (15ºC). At optimum temperature, only bands of moderate molecular weights were present reaching 53.4% at low osmotic water potential and moderate zinc concentration. Meanwhile at the temperature 30ºC, low and moderate molecular weight proteins were found at low osmotic water potential at all zinc concentrations. On the contrary, radicles of *S. occidentalis* showed relatively high molecular weight protein bands (207.1 KD) under different chosen treatments, particularly at low (15ºC) and high (30ºC) temperatures (Table 4). At high temperature, moderate molecular weight proteins were represented at a high percentage reaching 58.0% at all zinc concentrations. While at optimum temperature, a small proportion of moderate molecular weight bands was detected.

In desiccation, tolerant seeds, many of the qualitative differences in protein synthesis during development and germination are a consequence of the different enzymic processes on reserve accumulation and degradation, respectively [9,10]. Gel electrophoresis patterns of water soluble proteins showed that radicles *of S. alexandrina* and *S. italica* having low and moderate molecular weight proteins under all treatments. Sakova *et al*., [21] found no substantial differences between drought stressed and control plant patterns for SDS- protein. At high temperature, only moderate molecular weight proteins in radicles of *S. alexandrina*, existed with low osmotic water potential (at zinc concentrations 5 - 8 ppm), whereas in *S. italica* the same was true at optimum temperature. On the other hand, high molecular weight proteins were particularly found in radicles of *S. occidentalis* at 15 and 30ºC.

Generally, any interaction was particularly reflected in its effect on increasing the osmotic potential of the radicle in the three experimental plants under consideration which enhanced water uptake, thus facilitating enzymes activity and hydrolysis of reserve materials. Also, zinc addition apparently increased the translocation of water soluble metabolites to the radicle and later accumulation (allocation) of more reserve complex materials (soluble proteins) in the embryonic axis. This also helped the radicle capacity for water uptake and retention and consequently increased emergence, elongation and dry matter build up. Addition of zinc generally enhanced seed germination under arid and semi-arid conditions through increasing translocation of osmotically active metabolites as well as building up of reserve metabolites in the growing embryonic axis. Hence, the osmotic adjustment to decreasing water potential was particularly important to the function of radicle and later ensured transport of water to the hypocotyls.

Table (2): Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in the radicles of *Senna alexandrina*at different treatments of temperature (T), zinc (Zn) and osmotic water potential (φ_s).

T_{15}, ψ_{s0},Z n_0		**T_{15}, ψ_{s0},Zn_5**		**T_{15}, ψ_{s0}, n_8**		**T_{25}, ψ_{s1}, Zn_0**		**T_{25}, ψ_{s1}, Zn_5**		**T_{25}, ψ_{s1}, Zn_8**		**T_{30}, $\psi_{s0.7}$, Zn_0**		**T_{30}, ψ_{s1}, Zn_5**		**T_{30}, ψ_{s1}, Zn_8**	
M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%
110.1	8.4	110.1	8.4	112.5	7.5	111.4	6.4	118.9	8.9	117.2	7.1	—		118.9	8.9	117.1	7.1
91.2	7.2	91.2	7.2	90.2	6.2	96.1	6.9	96.9	6.9	94.1	6.0	94.9	19.9	96.9	6.9	96.1	6.1
86.6	3.6	86.6	3.6	84.6	3.6	85.1	5.1	83.9	3.9	84.2	4.2	84.1	4.1	83.9	3.9	84.1	4.2
72.2	3.1	72.2	3.1	—		—		—		—		—		—		—	
69.9	3.4	69.9	3.4	68.6	5.6	73.8	3.8	70.0	7.0	65.8	5.8	66.0	6.0	70.0	7.0	65.8	5.8
—		—		—		—		—		—		—		—		—	
—	4.8	—	4.8	50.1	5.1	45.9	5.9	49.2	4.2	45.5	5.5	45.3	5.3	49.2	4.2	50.5	5.5
31.3	7.7	31.3	7.7	28.6	8.6	35.7	5.7	45.6	5.6	27.4	7.4	35.9	5.9	—		—	
29.7	7.3	29.7	7.3	28.6	8.6	28.1	8.1	29.2	9.2	26.2	6.2	29.8	9.8	—		—	
—		—		—		25.4	9.4	—		—		—		—		—	
22.8	16.6	22.8	16.6	21.4	16.4	21.7	9.7	24.7	14.7	21.4	20.4	—		—		—	
19.8		19.8		—		—		—		—		20.4	15.8	—		—	
16.2	11.6	16.2	11.6	18.0	11.0	16.4	6.4	16.0	9.0	16.0	10.0	16.1	11.1	—		—	
—	9.4	—	9.4	14.0	9.1	14.2	8.2	14.3	8.3	14.0	20.0	14.5	8.5	—		—	
11.0		11.0		—		13.2	13.2	11.7	11.7	—		12.5	12.5	—		—	
9.1	11.3	9.1	11.3	10.9	10.9	—		—		—		—		—		—	

M. wt.= Molecular weight of soluble protein molecule.

Table (3): Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in the radicles of *Senna italica* at different treatments of temperature (T), zinc (Zn) and osmotic water potential (φ_s).

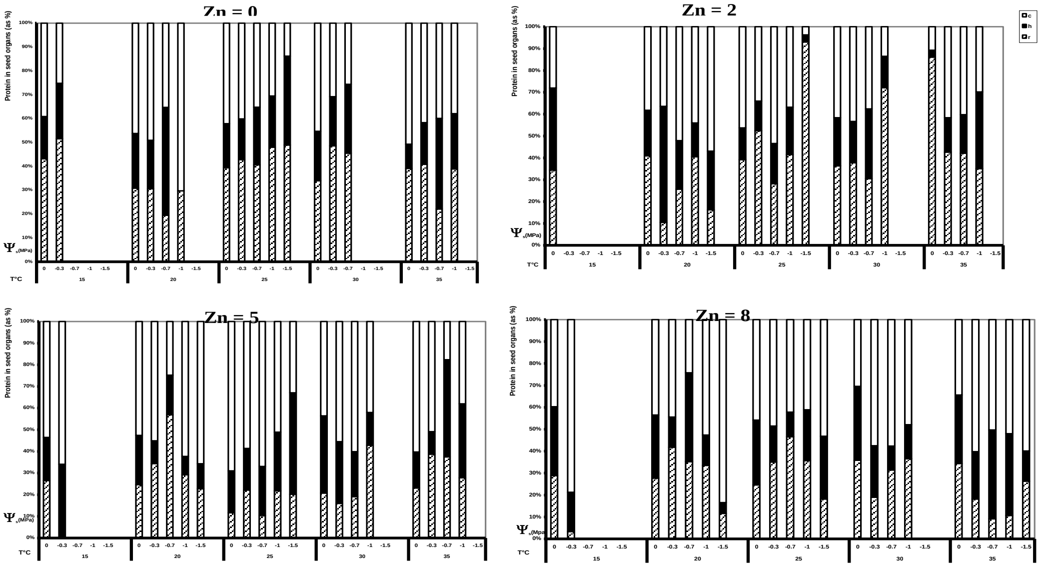
T_{15}, ψ_{s0}, Zn_0		**T_{15}, ψ_{s0}, Zn_5**		**T_{15}, ψ_{s0},, Zn_8**		**T_{25},ψ_{s1}, Zn_0**		**T_{25}, ψ_{s1},Zn_5**		**T_{25}, ψ_{s1}, Zn_8**		**T_{30}, ψ_1, Zn_0**		**T_{30}, ψ_{s1}, Zn_5**		**T_{30},ψ_{s1}, Zn_8**	
M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%
113.6	9.6	114.2	6.2	113.2	8.2	—		—		—		118.3	7.3	117.1	7.10	0	
92.8	6.7	93.0	5.9	93.7	6.7	—		—		—		96.8	6.8	96.1	6.3	99.8	7.9
83.3	3.3	83.0	3.3	83.2	4.2	—		—		86.5	5.5	83.8	3.4	84.1	4.2	84.2	4.1
—		74.9	4.6	75.7	5.7	74.9	24.8	—		77.3	17.3	—		—		—	
—	7.6	—	5.2	—	6.1	60.1	7.1	73.4	53.4	—		72.1	7.0	75.6	5.8	76.1	6.0
58.0	58.0	60.5	60.5	54.9	54.9	—		—		—		—		—			
—		—		—		—		—		—		54.2	4.2	55.5	5.5	55.4	5.3
34.6	4.6	34.8	4.8	34.9	4.9	—		—		—		35.5	7.5	37.4	7.4	35.9	5.9
28.2	3.2	—		28.1	1.2	—		—		—		29.2	9.2	26.2	6.2	29.8	9.8
—		—		—		—		—		—		—		—		—	
23.7	3.7	24.4	4.4	22.6	4.6	—		—		—		24.7	16.7	20.4	19.4	—	
—		—		—		—		—		—		—		—		19.4	14.3
—		—		—		—		—		—		19.0	9.1	18.9	10.0	17.0	12.1
—		—		—		—		—		—		14.3	7.3	14.0	24.0	14.5	9.6
—		—		—		—		—		—		11.7	12.7	—		12.4	22.5

M. wt.= Molecular weight of **soluble** protein molecule.

Table (4): Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in the radicles of *Senna occidentalis* at different treatments of temperature (T), zinc (Zn) and osmotic water potential (□$_s$).

T_{15}, ψ_{s0}, Zn_0		T_{15}, ψ_{s0}, Zn_5		T_{15}, ψ_{s0}, Zn_8		T_{25}, ψ_{s1}, Zn_0		T_{25}, $\psi_{s0.7}$, Zn_5		T_{25}, ψ_{s1}, Zn_8		T_{30}, $\psi_{s0.7}$, Zn_0		T_{30}, ψ_{s1}, Zn_5		T_{30}, ψ_{s1}, Zn_8	
M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%	M.wt	%
206.9		206.1	4.1	207.1	4.2	—		—		—		205.9	3.1	205.8	4.2	202.1	3.6
135.1	2.9	133.6	2.6	143.5	2.8	—		—		—		133.0	3.7	132.1	2.9	129.4	2.8
—	14.1	—	14.1	123.2	13.8	—		—		—		119.2	13.2	119.3	12.9	117.5	13.2
113.8		115.5		113.3		—		—		—		108.3	5.7	107.4	4.6	—	
—	6.2	—	5.8	—	7.3	—		—		—		—		—		—	
75.9		75.1		74.7		79.0	16.4	—		—		—		—		—	
75.8	15.4	78.6	25.5	77.6	24.2	74.3	17.3	—		75.0	24.8	—		—		—	
68.1	15.9	68.6	17.4	67.3	53.6	—		68.0	53.4	70.6	20.1	—		—		—	
59.4	19.9	—	6.3	—	9.2	—		—		—		61.1	54.9	62.2	60.5	62.6	58.0
49.3		46.4		46.6		—		—		—		—		—		—	
—		—	20.4	—	4.7	—		—		—		36.1	4.9	33.9	4.8	35.0	4.6
26.5	5.4	29.0		29.6		—		—		—		28.2	1.1	—		27.1	3.2
28.2	11.1	23.7	10.0	24.3	9.0	—		—		—		—		—		—	
23.2	8.6	—	9.0	—	8.3	—		—		—		22.6	4.6	22.7	4.4	22.8	3.7

M. wt.= Molecular weight of **soluble** protein molecule.



r = radicle

h = hypocotyl c = storage tissue

Figure (2): Relative distribution (as %) of soluble proteins in germinating *Sennaalexandrina* seed organs at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

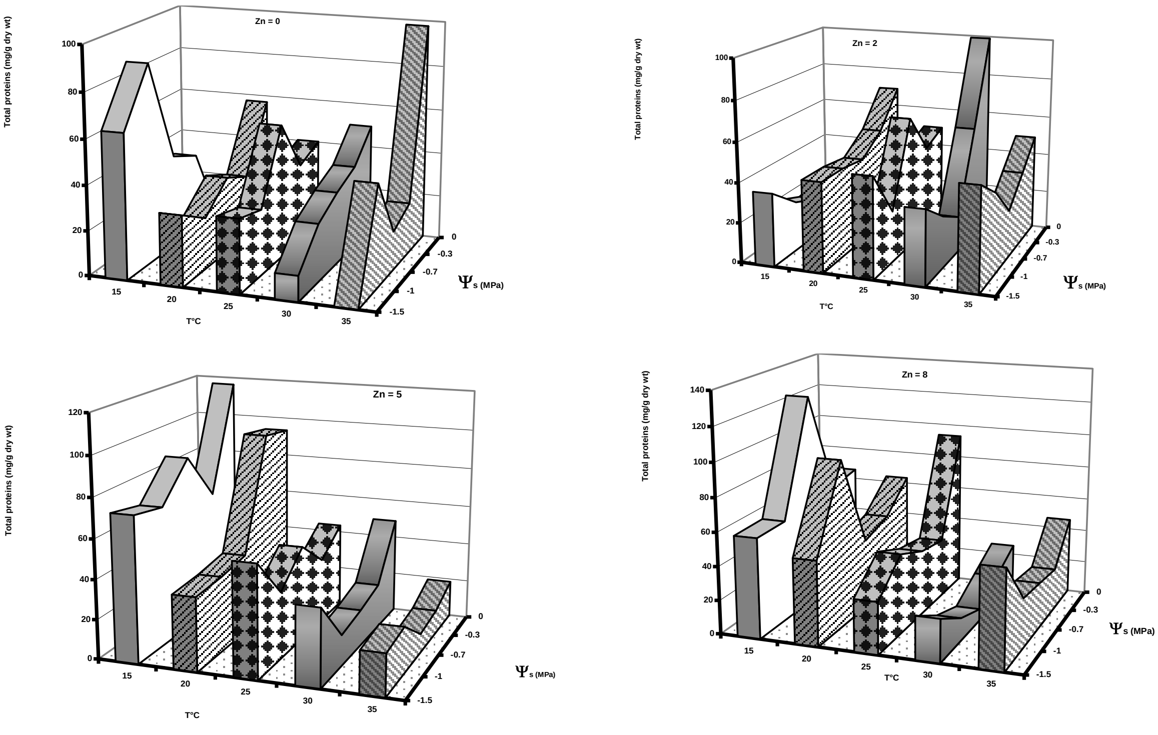
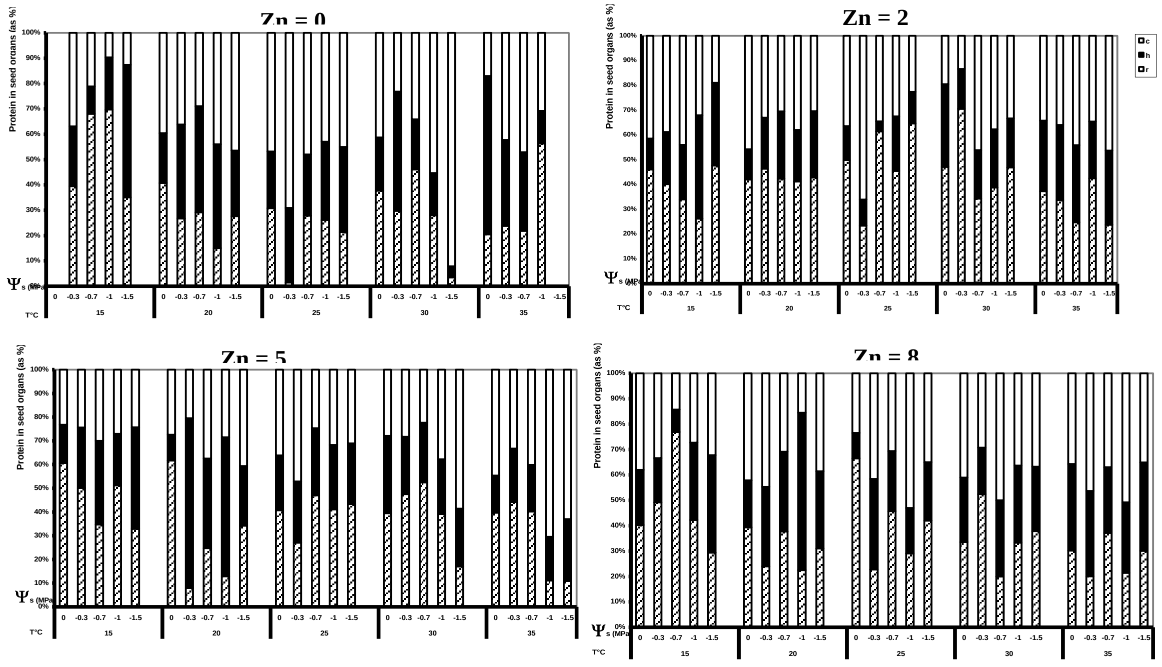


Figure (3): Total content of soluble proteins in germinating *Sennaitalica* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.



r = radicle h = hypocotyl c = storage tissue

Figure (4): Relative distribution (as %) of soluble proteins in germinating *Sennaitalica* seed organs at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.

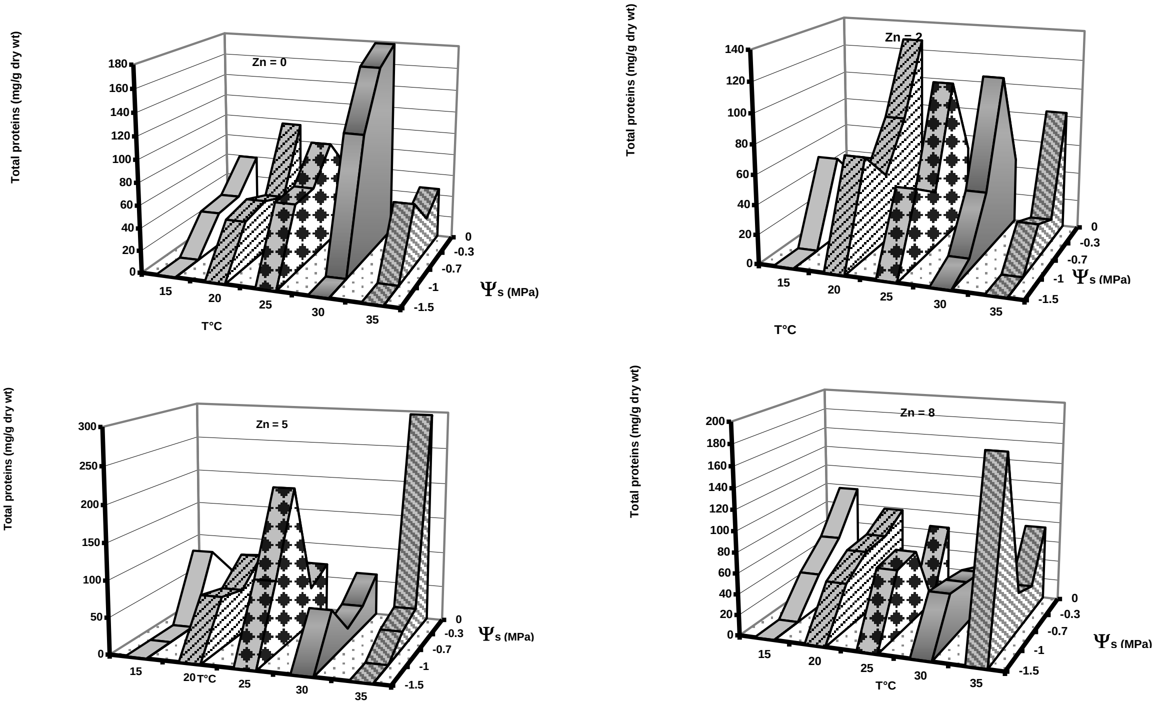
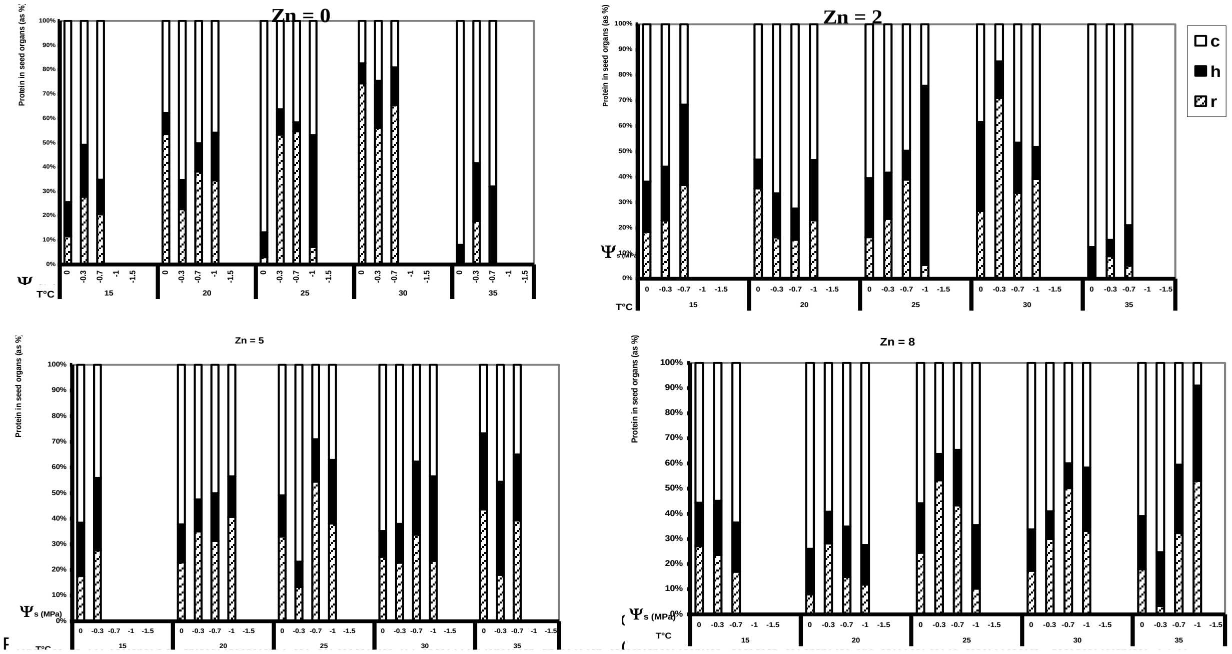


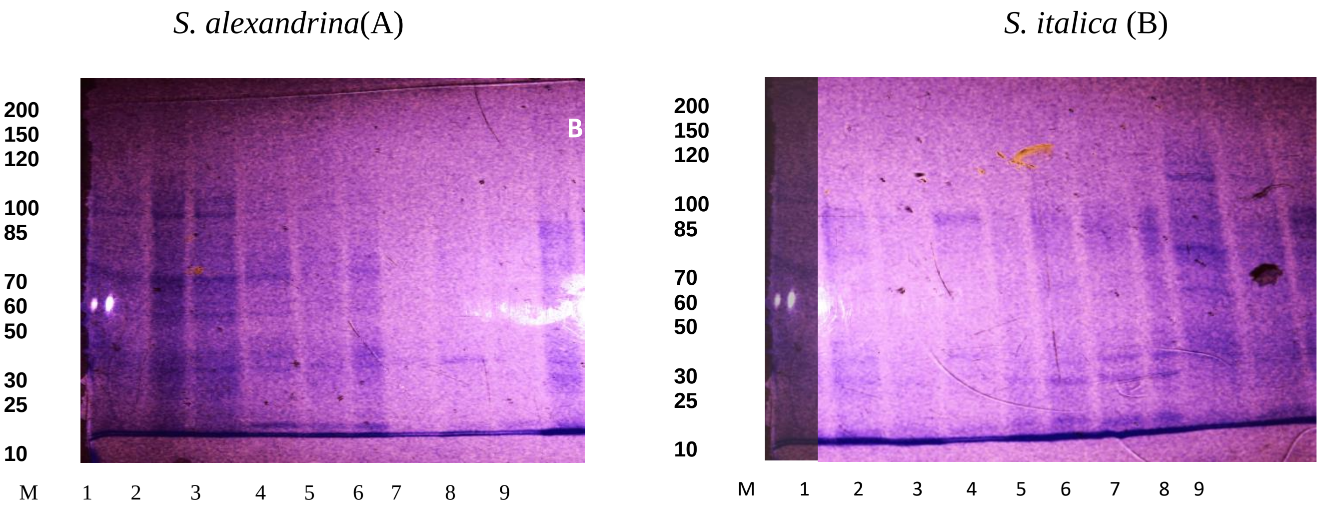
Figure (5): Total content ofsoluble proteins in germinating *Senna occidentalis* seeds at different osmotic water potentials (Ψ_s), temperatures (T) and zinc (Zn) concentrations.



r = radicle h = hypocotyl

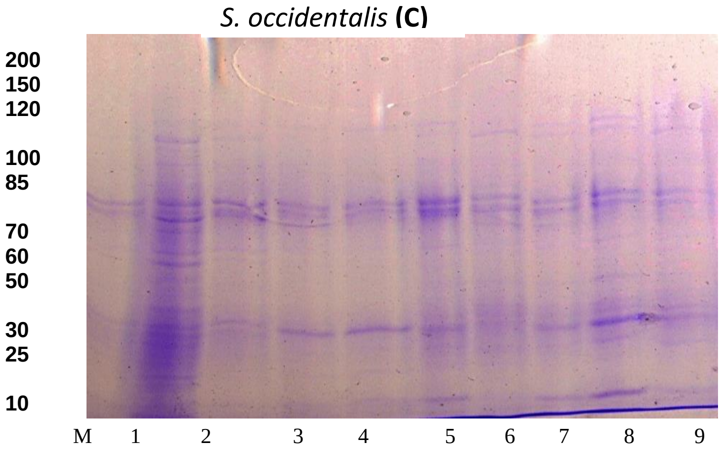
c = storage tissue

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



1 = T_{15}, ψ_{s0}, Zn_0 4 = T_{25}, ψ_{s1}, Zn_0 7 = T_{30}, $\psi_{s0.7}$, Zn_0
2 = T_{15}, ψ_{s0}, Zn_5 5 = T_{25}, ψ_{s1}, Zn_5 8 = T_{30}, ψ_{s1}, Zn_5
3 = T_{15}, ψ_{s0}, Zn_8 6 = T_{25}, ψ_{s1}, Zn_5 9 = T_{30}, ψ_{s1}, Zn_8

1 = T_{15}, ψ_{s0}, Zn_0 4 = T_{25}, ψ_{s1}, Zn_0 7 = T_{30}, ψ_{1}, Zn_0
2 = T_{15}, ψ_{s0}, Zn_5 5 = T_{25}, ψ_{s1}, Zn_5 8 = T_{30}, ψ_{s1}, Zn_5
3 = T_{15}, ψ_{s0}, Zn_8 6 = T_{25}, ψ_{s1}, Zn_8 9 = T_{30}, ψ_{s1}, Zn_8



1 = T_{15}, ψ_{s0}, Zn_0 4 = T_{25}, ψ_{s1}, Zn_0 7 = T_{30}, $\psi_{s0.7}$, Zn_0
2 = T_{15}, ψ_{s0}, Zn_5 5 = T_{25}, $\psi_{s0.7}$, Zn_5 8 = T_{30}, ψ_{s1}, Zn_5
3 = T_{15}, ψ_{s0}, Zn_8 6 = T_{25}, ψ_{s1}, Zn_8 9 = T_{30}, ψ_{s1}, Zn_8

Figure (7): Photoplates A, B & C: Soluble protein patterns (Coomassie blue stain 12% SDS-PAGE gel) in the radicles of the three species investigated at different treatments (temperature (T), osmotic water potential (Ψ_s) and zinc (Zn)).

4 Conclusion

Finally, zinc addition induced adaptation to suboptimal temperatures through increasing the total amounts of metabolites (such as soluble proteins) in organs of the growing *Senna* seedlings. The germinating radicles of seeds in *S. occidentalis* (wild mesophyte) were characterized by the presence of high molecular weight protein bands, whereas radicles of xerophytes contain low molecular weight protein bands. Furthermore, the statistical analysis indicated that the trifactorial interaction (ΨsxTxZn) had the major effect on the soluble proteins in different organs of the three-plant species. Exceptionally, the effect of osmotic water potential was the dominant in storage tissue proteins of *S. occidentalis*.

5 References

[1] I. Cakmak: Role of zinc in protecting plant cells from reactive oxygen species. *New Phytologist*, 146:185–205, (2000).

[2] H. M. El-Sharkawi and I.V. Springuel: Germination of some crop plant seeds under reduced water potential. *Seed Science and Technology*, 5:677-688, (1977).

[3] H. M. El-Sharkawi, and K. A. Farghali: Interactive effects of water potential and temperature in the germination of seeds of three desert perennials. *Seed Science and Technology*, 13: 265-285, (1985)

[4] H. M., El-Sharkawi, K. A. Farghali, and S. A. Sayed: Growth characteristics of *Triticum aestivum*L. roots under different treatment combinations of boron, matric water potential and temperature. *Seed Science and Technology*, 27: 239-249, (1999).

[5] K. A. Farghali and A.A. El-Aidarous Thermostability of chlorophylls in some native species of xerophytes. *IOSR Journal of Agriculture and Veterinary Science*. 6(6)- 52-65, (2014).

[6] Q .Fu, ; B. C. Wang, X. Jin, H. B Li, P. Han , K.H Wei, . X. M .Zhang , . and Y.X. Zhu.: Proteomic analysis and extensive protein identification from dry, germinating *Arabidopsis* seeds and young seedlings. *Journal of Biochemistry and Molecular Biology*, 38(6):.650-660, (2005).

[7] J. F. C. Gonçalves; A. V. Fernandes; A. F. M. Oliveira; L. F. Rodrigues and R. A. Marenco: Primary metabolism components of seeds from Brazilian Amazon tree species. *Brazilian Journal of Plant Physiology*, 14(2):139-142, (2002).

[8] C. Gonzalez-Murua, M. Sanchez-Diaz, P. Aparicio-Tejo, , A. Munoz-Rueda, and J. S. G. Reid: The effect of NaCl and water stress on germination and α-galactosidase activity in germinated seeds of *Medicago sativa,; Trifolium repens* and *T. brachycalycinum*. *Journal of Plant Physiology*119, 317–326, (1985).

[9] A. R. Kermodeand , J.D. Bewley: The role of maturation drying in the transition from seed development to germination. II. Post-germinative enzyme production and soluble protein synthetic changes within the endosperm of *Ricinus communis* L. seeds. *Journal of Experimental Botany*, 36: 1916-1927, (1985).

[10] A. R. Kermode and J. D. Bewley: Alteration of genetically regulated syntheses in developing seeds by desiccation. In: AC Leopold, ed. Membranes, Metabolism and Dry Organisms, 59-84. *Ithaca: Cornell University Press* 24, (1986).

[11] U. K. Laemmli: Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 227, 680–685,(1970).

[12] F. M. Lai; C. G. Lecouteux, and B. D. McKersie: Germination of alfalfa (*Medicago sativa* L.) seeds and desiccated somatic embryos. 1-Mobilization of storage reserves. *Journal of Plant Physiology*, 145:507-513, (1995).

[13] R. B. S. Lima, J. F. C.Gonçalves, S. C. Pando; A.V. Fernandes, and A. L.W. Santos: Primary metabolite mobilization during germination in rosewood (*Anibarosa eodora* Ducke) seeds. *R. Árvore, Viçosa-MG*, 32:19-25, (2008).

[14] A. Llanes, H. Reinoso, and V. Luna: Germination and early growth of *Prosopis strombulifera* seedlings in different saline solutions. *World Journal of Agricultural Sciences*, 1 (2): 120-128, (2005).

[15] C. H. Lowry; N.K. Rosebrought, A.L. Farrand, R.J. Randall: Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193:256-275, (1951).

[16] S. P. McGrath; M. Fan and F. Zhao: Decreased zinc concentrations in wheat grain are due to increased yield and harvest index. In: Conf. Proc.: Zinc Crops Improving Crop Production and Human Health, Istanbul, Turkey, (2007).

[17] S. Nein and S. P. Vyas, : Seed germination, seedling growth and nitrogen metabolism of cluster bean genotypes as affected by hyperthermia. Advances in arid legumes research. Henry,A., Kumar, D., Singh, N.B.(eds). Regional Research Station, Central Arid Zone Research Institute, Kukma-370 105 Bhuj (Gujarat), India, (2003).

[18] C. Nozillo; P. J. Lea and F. A. Loewus: Recent advances in phytochemistry mobilization of reserves in germination. *Plenum Press, New York*, 17: 77-109, (1983).

[19] B. Ostle: Statistics in research. Iowa State University Press, Ames. Pp. 585. (1963).

[20] N. A. Ploxinski,: Rucovodstropobiometriidlyazootexnikov. Izdatel' stvo "Kolos" Moskow. (1969)

[21] L. Sakova; V. Curn and M. Novaka: Use of biochemical genetic markers for testing changes in protein spectra following drought stress in legumes. Sbmike-JihoceskaUniverziteZernedeleskaFakulte Ceske Budejovice FytotechnickaRada, 14(1): 65-74, (1997).

[22] A. F. Tossi, and B.H. Bakar: Effects of light, temperature and different media on seed germination of *Brassica juncea*(L. Czern) var. Ensabi in the laboratory. .*Journal of Food, Agriculture & Environment*, 5 (3&4) : 258-260,(2007).

[23] B. L. Vallee and F K.H. alchuk: The biochemical basis of zinc physiology. *Physiological Reviews*, 73: 79-118, (1993).