Interactive effect of drought and calcium chloride treatments on growth criteria and some metabolic activities of *Vigna membranacea*

H. M. Mandururah & A. A. Alayafi

Department of Biological Sciences, Girls Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
Corresponding Author: aal_shareaf@hotmail.com

ABSTRACT: Drought is the most important environmental factors limiting crop productivity.This study aimed at evaluating the effects of varying soil moistures on (*Vigna membranacea*) seeds with this compound. (*Vigna membranacea*) seeds were subjected to each of the following treatments: a) soaked in distilled water for 6 hours, b) soaked in $\mathbf{10^{-4},10^{-3},10^{-2}}$ $mMCaCl_2$ for 6 hours, Plants after 21 days were subjected to drought for 15 days, after which lot of the stressed plants were harvested, the remaining stressed plants were irrigated and harvested 4 hours later, in order to provide a set of recovering plants..Maximum germination percentages of seeds attained in case of seeds soaked for 6 hours after their exposure to $CaCl_2$ concentration, Results showed that in general that presoaking of seeds in $CaCl_2$ solutions ($\mathbf{10^{-4}}$, $\mathbf{10^{-3}}$, $\mathbf{10^{-2}}$) reduced the inhibitory effect of water stress on root and stem growth and makes plant more drought –tolerant. Such effects were reflected on the increased leaf growth parameter, leaves content of photosynthetic pigments of plants,enhancement of total soluble sugars, free amino acids and proline contents. On the other hand, hardening enhances an increase in carbohydrate contents. The results also indicated that the stimulated activities of amylase and peroxidase is well correlated with the decrease of $CaCl_2$ concentrations.

[H. M. Mandururah & A. A. Alayafi. **Interactive effect of drought and calcium chloride treatments on growth criteria and some metabolic activities of *Vigna membranacea*.** *Life Sci J* 2015;12(2):124-132]. (ISSN:1097-8135). http://www.lifesciencesite.com. 18

Key words: Vigna membranacea seeds, drought treatment, growth parameters

INTRODUCTION

Drought is the most important environmental factors limiting crop productivity particularly in temperate regions (Farooq et al, 2009).

Generally, water deficit reduced the growth of drought sensitive plants. Reduction in plant growth by water deficit may result from the disturbance of water balance, reduction of leaf expansion and elongation, mitotic division of cells, fresh and dry weights, changes in various physiological and metabolic processes (such as decline in solute) and in antioxidant accumulation and expression of stress specific genes (Ron&Eduardo,2010;Wim et al. 2013; Rosa et al. 2014).

It has been reported that seed treatment with some compounds such as calcium (Chengbin *et al.* 2013), vitamins and organic acids (Hassanein et al, 2009) prevente to some extent the inhibitory effects of water or salt stress.

The aim of this investigation was to study how plant growth and the important cell constituents such as nitrogenous components, carbohydrates, photosynthetic pigments and some enzyme activities in *Vigna membranacea* plant is altered during exposure to varying soil moistures.

Presoaking of seeds in different concentration of $\mathbf{CaCl_2}$ was included in the study to see their interactive effect with drought and therefore to ascertain whether these concentration can alleviate or modify the pattern of changes induced by water deficit.

Vigna membranacea seeds was chosen in this study because of their importance as valuable crops. The seeds are good source for protein, carbohydrates, and contain active substances which are used in medicine. The root of plant live symbiotically with nodular bacteria, which can fix the atmospheric nitrogen and thus positively contribute to nitrogen balance of the cropping system (Marie et al,2013).

Materials & Methods

To achieve the previous objectives the following experiments were run:-

1- Seeds were subjected to each of the following treatments:-
    a- Soaked in distilled water for 6 hours
    b- Soaked in 1^{0-4}, 1^{0-3}, 1^{0-2} $CaCl_2$ for 6 hours.
2- Seeds were germinated in plastic pots filled with vermiculite and received similar growth conditions. Pots were irrigated with distilled water for one week until the completion of

seedling emergence, then with one quarter strength Hogland nutrient solution until the end of 21 days after which, the plants were subjected to drought for 15 days. The seedlings were divided into 4 groups watered according to the level of vermiculite moisture content starting from water capacity of 100% then 75 %,45%, 35 %,and were subjected to drought for 15 days after which a lot of stressed plants were harvested.The remaining stressed plants were irrigated with 100% of water capacity and harvested 4 hours later, in order to provide a set of recovering plants.

3- Parameters selected to evaluate the effects of previous treatments were:-
 Growth parameter, estimation of plant photosynthetic pigments (Lichtenthaler, 1987), carbohydrate content(Naguib,1964), free amino acids (Naguib,1969), protein (Hatree, 1972) and praline(Bates et al.,1977), activity of some enzymes; amylase(Das and Sen-Mandi,1992) and peroxidase (Kar and Mishra, 1976).

Results & Discussion

Many studies have been made concerning the effect of water deficit on seed germination, Hadas (1976) reported that retardation in germination is related to the serious effect of low water potential on the enzymes activities. On other hand, Dubey & Sharma (1990) pointed out that water stress limited the hydrolysis of food reserves in the endosperm of rice & this prevented the translocation of soluble materials to the developing embryos.

The preliminary results in the present investigation revealed that maximum germination percentages of seeds attained in case of seeds soaked for 6 hours after their exposure to $CaCl_2$ concentrations. The length of the radical, the number of secondary roots and hypocotyls length were extremely increased due to $CaCl_2$ – seeds treatment at 10^{-4}, 10^{-3}, and 10^{-2} mM.

It can be noticed in general, that pre-soaking of seeds in $CaCl_2$ solution reduced the inhibitory effect of water stress on root stem growth. The plant response was not constant throughout the stress period.

The morphological characters of a plant are dependent on its rate of growth, particularly under water stress. Under the prevailing experimental conditions, the leaf area, leaf fresh and dry weight of plants grown from -$CaCl_2$ – soaked seeds were less affected by water stress compared with those grown from unstressed plants (Table1a). Comparison of unstressed plant and stressed plant showed that the percentages of dry matter was less and LAR (leaf area ratio) was greater (except, in case of 10^{-2} mM $CaCl_2$**)** in seedling grown from treated seeds under unstressed condition (table 1b-c). The opposite was true in the stressed plants. The values of dry matter percentages were affected only slightly at the moderate water capacity and the difference were not statistically significant. In the most of the stressed treatments, dry matter percentage were further increased up to 17% in $CaCl_2$ treated seedlings of the control treatment.

Table (1a): Impact of different calcium chloride concentration on the growth parameters of the seedling of soaked seeds of *Vigna membranacea*. (No water stress regimen were applied)

$Cacl_2$ CO.(mM)	Leaf area cm^2	Leaf metric ratio			Weights (g)					
		leaf area ratio (LAR)	specific leaf weight (SLA)	leaf weight ratio (LWR)	leaf		stem		root	
					Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0	18.73	2.02	14.40	0.14	0.081	0.013	0.49	0.057	0.38	0.022
10^{-4}	15.20	2.53	25.33	0.10	0.045	0.006	0.47	0.043	0.22	0.014
10^{-3}	18.41	2.37	26.30	0.09	0.052	0.007	0.55	0.057	0.27	0.016
10^{-2}	12.51	1.50	25.02	0.06	0.035	0.005	0.39	0.048	0.67	0.038

* Significant at 5%; ** Significant at 1%

Table (1b): Impact of different calcium chloride concentration on the growth parameters of the seedling of soaked seeds of *Vigna membranacea.* (No water stress regimen were applied)

* Significant at 5%; ** Significant at 1%

Cacl$_2$ CO .(mM)	Water capacity %	Leaf area Cm^2	Leaf parameters ratio			Weights (g)					
			leaf area ratio (LAR)	specific leaf weight (SLA)	leaf weight ratio(LWR)	leaf		stem		root	
						fresh	dry	fresh	dry	fresh	dry
0	100	20.13	0.84	16.78	0.05	0.081	0.012	1.20	0.175	0.88	0.061
	75	19.78	1.15	16.48	0.07	0.083	0.012	0.86	0.128	0.66	0.042
	45	19.62	1.15	16.35	0.07	0.071	0.012	0.77	0.111	0.63	0.049
	35	17.43	0.75	12.45	0.06	0.087	0.014	0.83	0.128	1.12	0.085
10^{-4}	100	22.14	1.27	15.81*	0.08	0.086	0.014*	0.84	0.115	0.50	0.037
	75	19.79	1,26	17.99*	0.07	0.079	0.011*	0.94	0.135	0.48	0.034
	45	20.46	1.12	18.60*	0.06	0.076	0.011*	0.79	0.111	0.65	0.057
	35	19.86	1.32	16,55*	0.08	0.074	0.012*	0.71	0.115	0.39	0.031
10^{-3}	100	22.30	0.92	13.12	0.09	0.091	0.017	0.97	0.125	0.47	0.038
	75	19.15	0.96	13.68	0.07	0.077	0.014	0.86	0.114	0.48	0.042
	45	22.17	0.92	18.47	0.05	0.072	0.012	0.99	0.167	0.76	0.072
	35	19.49	1.20	14.99	0.08	0.080	0.013	0.75	0.113	0.61	0.041
10^{-2}	100	20.82	0.91	13.01	0.07	0.102	0.016	1.09	0.172	0.88	0.055
	75	25.72	1.20	17.15	0.07	0.098	0.015	1.18	0.173	0.64	0.045
	45	20.03	1,11	22.26	0.05	0.076	0.009	1.09	0.145	0.66	0.058
	35	18.66	0.86	14.35	0.06	0.086	0.013	0.87	0.120	0.47	0.037

There are much evidences in the literature suggesting that the composition and function of the photosynthetic apparatus of plants undergo marked changes in response to water stress (Michelle et *al*. 2014). It was noted in this investigation that the photosynthetic pigments (table 2) of plants treated with $CaCl_2$ showed considerable decreased compared to the contents of treated unstressed plants. The post stress recovery of pigment level was observed with respect to the recovery of water stress. This induction being most marked in $CaCl_2$ treated seedlings, the chlorophyll a/b ratio showed tendency increased or

No significant decrease with stress conditions. It is pertinent to presume that $CaCl_2$ has delayed leaf senescence of stressed seedlings by checking the decline in chlorophyll in comparison to untreated stressed plants. The reduction in total photosynthetic pigments has been reported to be related to the activation of chlorophyllase, which catalyses the catabolism of chlorophyll (Vaz *et al*., 2010). Concerning the transit changes in carbohydrate constituents under drought stress, the results showed that all carbohydrate constituents in the shoot and roots particularly soluble sugars (reducing and non reducing sugars) were markedly enhanced in response to drought treatments. (tables 3a&b), The increase in starch content in shoots of stressed seedlings (which grown from treated $CaCl_2$ seeds) due to the stimulatory effect on its synthesis. This could plays to some extent, a role in osmo-regulation and cell turgidity.

In accordance to this view, the accumulation of soluble sugars has been considered of importance for the adjustment of cellular water potential under conditions of water deficit and may be regarded as nonionic osmo regulatory agent,resulting in increased cell turgidity (Kim *at al*,2000;Pinheriro *et al*,2011).The increase in starch content in shoot of stressed seedlings (which grown from $CaCl_2$ treated seeds), may be related to the inhibitory effect of hardening treatments on starch degradation or due to the stimulatory effect on its synthesis.

Table (1c): Impact of different calcium chloride concentration on the growth parameters of the seedling of soaked seeds of *Vigna membranacea*. (after four hours of water stress application)

$Cacl_2$ CO. (mM)	Water capacity %	Leaf area Cm^2	Leaf parameters ratio			Weights (g)					
			leaf area ratio (LAR)	specific leaf weight (SLA)	leaf weight ratio (LWR)	leaf		stem		root	
						fresh	dry	fresh	dry	fresh	dry
0	100	21.74	1.19	19.76	0.06	0.081	0.011	0.94	0.129	0.51	0.049
	75	18.57	0.84	16.88	0.05	0.073	0.011	1.32	0.179	0.41	0.042
	45	19.17	1.05	17.43	0.06	0.080	0.011	0.83	0.121	0.51	0.050
	35	16.90	1.23	15.36	0.08	0.074	0.011	0.62	0.084	0.46	0.047
10^{-4}	100	20.45	1.30	18.59**	0.07	0.070	0.011*	0.80	0.102	0.39*	0.038
	75	17.68	1.18	19.64**	0.06	0.071	0.009*	0.83	0.095	0.35*	0.035
	45	14.89	1.28	21.27**	0.06	0.049	0.007*	0.69	0.092	0.28*	0.023
	35	16.38	1.09	18.20	0.06	0.056	0.009*	0.70	0.101	0.31*	0.033
10^{-3}	100	20.49	1.37	17.08	0.08	0.092**	0.012*	0.85	0.115	0.36	0.032
	75	19.61	1.53	21.79	0.07	0.050**	0.009*	0.79	0.095	0.24	0.021
	45	18.98	1.05	21.09**	0.05	0.053**	0.009*	0.77	0.110	0.61	0.063
	35	15.46	1.16	19.33**	0.06	0.050**	0.008*	0.87	0.107	0.29	0.025
10^{-2}	100	22.18	1.37	17.06*	0.08	0.075	0.013	1.04	0.127	0.32	0.031
	75	22.28	1.24	24.76*	0.05	0.062	0.009	0.88	0.124	0.37	0.042
	45	22.97	1.15	22.97*	0.05	0.069	0.010	1.07	0.164	0.51	0.049
	35	15.62	1.30	26.03*	0.06	0.042	0.006	0.62	0.081	0.33	0.029

* Significant at 5%; ** Significant at 1%

Under water stress conditions, some nitrogenous components increase in plant tissues (Peleh *et al*., 1997), while in others decrease (Harrak *et al*., 1999). The free amino acid content recorded in (table 4) showed a markedly decrease under drought stress, and almost increased by increasing $CaCl_2$ concentrations at each level of water capacity, though the effect was more pronounced for those seedlings of treated seeds when they were compared with the control (seedlings of untreated seeds). Plants grown from water - soaked seeds recorded reduction in their protein content with the decrease in water capacity, although exhibited relatively higher values than the other treatments (except the 10^{-2} $CaCl_2$ treatment for 45% and 35% water capacity). On the other hand, hardening resulted in an increase of the protein content of seedlings compared to unstressed plants of water soaked seeds (table 5). This runs parallel with the increase in carbohydrate contents (total available sugars). It is reported that the increase in proteolytic activities resulted in loss of protein under water stress (Shi-Lin *et al*, 2014 & Marjetka *et al*, 2014). The accumulation of amino acids and proline in drought stressed plants may have resulted from protein degradation and also indicates the inhibition of incorporation of amino acid into polypeptide chains Santiago *et al,* 2013).

Table (2): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on the on the content of photosynthetic pigments of the seedlings.

Treatments	Water capacity %	$Cacl_2$ CO.(mM)														
		0					10^{-4}					10^{-3}				
		Chl.a Mg/g/fresh wt.	Chl.b Mg/g/fresh wt.	Cart. Mg/g/fresh wt.	Total	a\b	Chl.a Mg/g/fresh wt.	Chl.b	Cart.	Total	a\b	Chl.a	Chl.b	Cart.	Total	a\b
Before water stress	100	1.81	0.56	0.71	3.08	3.23	2.20	0.61	0.79	3.60	3.61	1.93	0.52	0.70	3.15	3.71
During water stress	100	1.47	0.44	0.61	2.52	3.34	1.48	0.41	0.64	2.53	3.61	1.27	0.47	0.67	2.41	2.70
	75	1.42	0.41	0.62	2.45	3.46	1.46	0.35	0.55	2.36	4.17	1.41	0.44	0.59	2.44	3.20
	45	1.30	0.38	0.52	2.20	3.42	1.07	0.33	0.47	1.87	3.24	1.37	0.42	0.60	2.39	3.26
	35	1.08	0.35	0.48	1.91	3.09	1.04	0.31	0.46	1.81	3.35	1.11	0.32	0.58	2.01	3.47
after four hours of water stress)	100	1.74*	0.59 **	0.78**	3.11**	2.95	2.14**	0.62**	0.64*	3.40 **	3.45	2.18**	0.69**	0.91**	3.78**	3.16
	75	1.90*	0.59 **	0.82**	3.31**	3.22	2.60**	0.60**	0.70*	3.90 **	4.33	2.15**	0.62**	0.88**	3.65**	3.47
	45	2.34*	0.66 **	0.74**	3.74**	3.55	1.62**	0.56**	0.88*	3.06 **	2.89	2.11**	0.60**	0.87**	3.58**	3.52
	35	1.92*	0.56 **	0.67**	3.15**	3.43	1.84**	0.53**	0.91*	3.28 **	3.47	1.74**	0.56**	0.93**	3.23**	3.11

Cont.

Treatments		Cacl$_2$ CO.(mM)				
		10-2				
	Water capacity %	Chl.a	Chl.b	Cart.	a\b	Total
Before water stress	100	1.58	0.59	0.55	2.72	2.68
During water stress	100	1.15	0.35	0.53	2.03	3.29
	75	1.41	0.36	0.60	2.37	3.92
	45	0.96	0.27	0.56	1.55	3.56
	35	0.80	0.25	0.50	4.58	3.20
after four hours of water stress)	100	2.35**	0.72**	1.51*	4.58**	3.26
	75	2.09**	0.78**	1.72*	4.59**	2.68
	45	1.91**	0.56**	0.87*	3.34**	3.41
	35	1.43**	0.63**	0.97*	3.03**	2.27

Table (3a): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on root carbohydrates content (mg sugar\g d.w.)

Treatment	Water capacity %	Cacl$_2$ CO.(mM)											
		0				10-4				10-3			
		Reducing sugars	Non Reducing sugars	Starch	Total sugars	Reducing sugars	Non Reducing sugars	Starch	Total sugars	Reducing sugars	Non Reducing sugars	Starch	Total sugars
Before water stress	100	5.15	48.51	94.44	148.10	1.56	33.44	105.99	140.99	1.99	35.66	93.78	131.43
During water stress	100	3.37	59.73	120.78	183.88	6.96	44.03	131.55	182.54	4.59*	52.31*	147.11**	204.01 **
	75	17.96	84.44	129.33	231.73	15.44	40.33	130.67	186.44	27.99*	64.48*	154.89**	247.36 **
	45	23.96	91.14	145.67	260.77	16.41	45.63	125.78	187.82	30.33*	81.18*	173.99**	285.50 **
	35	31.23	111.46	148.99	291.68	35.66	43.18	129.89	208.73	33.29*	96.18*	137.99**	267.46 **
after four hours of water stress	100	5.43*	67.83	129.11*	202.37	2.85	67.14 **	121.22	191.21*	1.78	47.18	109.22	158.18
	75	24.54*	62.25	140.99*	227.78	9.35	59.07**	127.61	196.03*	12.15	47.97	111.22	171.29
	45	33.44*	66.90	157.22*	257.56	30.18	74.03**	123.89	228.10 *	8.52	51.03	133.67	193.22
	35	45.87*	74.62	178.72*	299.21	25.11	67.10**	127.99	220.20 *	4.11	60.14	124.22	188.47

cont.

Treatment	Water capacity %	Cacl$_2$ CO.(mM)			
		10^{-2}			
		Total sugars	Starch	Non Reducing sugar	Reducing sugars
Before water stress	100	173.7	108.11	44.48	21.11
During water stress	100	228.8	167.66*	35.07**	26.02**
	75	268.1	210.22*	23.99**	33.85**
	45	209.2	149.11*	22.04**	38.03**
	35	206.4	137.67*	27.94**	40.83**
after four hours of water stress	100	197.2	118.22	65.55	13.44
	75	183.3	113.33	62.92	7.07
	45	220.9	137.89	58.48	24.55
	35	185.5	112.55	62.51	10.41

Table (3b): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on shoot carbohydrates content (mg sugar\g d.w.)

Treatment	Water capacity %	Cacl$_2$ CO.(mM)											
		0				10-4				10-3			
		Reducing sugars	Non Reducing sugars	Starch	Total sugars	Reducing sugars	Non Reducing sugars	Starch	Total sugars	Reducing sugars	Non Reducing sugars	Starch	Total sugars
Before water stress	100	28.41	41.98	139.78	210.17	3.81	44.29	111.67	159.77	3.19	42.88	111.44	157.51
During water stress	100	44.95	49.65	186.22	280.82	58.33	22.18	142.67	223.18	40.99	49.29	159.33*	249.61*
	75	49.77	65.98	192.22	307.97	49.74	31.70	152.22	233.66	53.76	64.81	172.99*	291.56*
	45	61.64	85.94	229.33	376.91	51.11	37.92	153.33	242.36	63.22	84.58	221.72*	369.52*
	35	71.03	100.40	239.66	411.09	68.77	41.92	150.22	260.91	57.22	114.06	195.44*	366.72 *
after four hours of water stress	100	41.44	68.63	203.55*	313.62	52.57	60.88**	138.89*	252.34	29.53	57.66	145.33	232.52
	75	59.51	69.55	263.11*	392.17	46.52	59.14**	134.22*	239.88	31.33	42.26	150..99	224.58
	45	62.77	75,36	257.66*	395.79	59.77	80.36**	140.89*	281.02	69.20	68.65	184.78	322.63
	35	71.66	95.14	254.11*	420.91	67.99	77.84**	146.99*	292.82	41.39	59.18	126.99	227.56

Cont.

Treatment	Water capacity %	Cacl$_2$ CO.(mM)			
		10^{-2}			
		Total sugars	Starch	Non Reducing sugar	Reducing sugars
Before water stress	100	257.13	164.55	32.77	59.81
During water stress	100	300.21	208.55*	32.74	58.92
	75	320.36	222.11*	37.49	60.76
	45	324.75	216.11*	45.09	63.55
	35	267.12	151.66*	31.13	84.33
after four hours of water stress	100	260.71	172.94	37.03*	50.74
	75	265.72	149.78	64.28*	51.66
	45	328.29	172.99	70.99*	84.31
	35	260.74	144.99	42.22*	73.53

Table (4): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on root and shoot amino acids content (mg \g d.w.)

Treatment	Water capacity %	Cacl$_2$ CO.(mM)											
		0			10^{-4}			10^{-3}			10^{-2}		
		Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
before water stress	100	12.64	17.67	30.31	7.00	20.84	27.84	9.64	13.67	23.31	11.67	17.89	29.56
During water stress	100	3.89	14.64	18.53	11.86	17.41	29.27*	12.97*	42.99	55.96*	21.02**	24.33*	45.35**
	75	3.64	4.11	7.75	6.47	26.19	32.66*	11.58*	29.22	40.8*	23.91**	25.83*	49.74**
	45	2.42	4.47	6.89	6.08	15.57	21.65*	9.31*	18.03	27.34*	20.28**	31.03*	51.31**
	35	5.89	4.25	10.14	8.86	13.49	22.35*	21.96*	25.04	47.00*	18.11**	20.17*	38.28**
after four hours of water stress	100	6.30*	13.19*	19.49*	4.94	14.49	19.43	4.39	15.55	19.94	6.25	21.30	27.55
	75	4.78*	20.25*	25.03*	6.92	13.03	19.95	9.69	21.22	30.91	4.94	15.44	20.38
	45	4.72*	20.05*	24.77*	7.28	14.00	21.28	1.58	11.51	13.09	3.36	11.33	14.69
	35	6.31*	16.14*	22.45*	5.42	10.11	15.53	7.36	9.06	16.42	4.97	11.22	16.19

Table (5): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on root and shoot proteins content (mg \g d.w.)

Treatment	Water capacity %	$CaCl_2$ CO.(mM)											
		0			10^{-4}			10^{-3}			10^{-2}		
		Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total
Before water stress	100	48.06	81. 62	129.68	49.78	78. 87	128.65	60.61	94.67	155.28	40.56	78.94	119.50
During water stress	100	40.72	84.84*	125.56*	33.39 **	48.92	82.31**	38.56	59.17**	97.73*	35.94**	51.89**	87.83**
	75	48.89	74.34*	123.23*	37.44 **	51.28	88.72**	45.42	61.06**	106.48*	43.56**	59.61**	103.17**
	45	33.55	69.61*	103.16*	41.94 **	52.33	94.27**	36.89	61.28**	98.17*	43.22**	71.39**	114.61**
	35	37.89	70.95*	109.84*	47.11 **	55.78	102.89 **	42.78	55.33**	98.11*	54.22**	68.17**	112.39**
after four hours of water stress	100	45.50	85.22	130.72	25.27	39.77	65.04	37.99	52.22	90.21	21.66	30.72	52.38
	75	22.88	29.72	52.60	29.11	44.66	73.77	34.55	41.16	75.71	21.44	27.05	48.49
	45	18.61	29.36	47.97	28.83	35.99	64.82	35.22	45.83	81.05	12.83	29.61	42.44
	35	23.88	29.83	53.71	27.49	39.44	66.93	35.83	38.83	74.66	27.83	40.61	68.44

The accumulation of amino acids and proline, under drought stress may play a role in osmo regulation and serve as available source of carbon and nitrogen. In addition, proline also serves as a hydroxy radical scavenger and as a means of reducing the acidity in the cell (Thangella et al, 2013).

Seed-hardening treatments resulted in significant increase of proline contents (Table 6) the increase in proline content was proportional to the decrease in the applied water capacity.

Under drought stress, the amylase and peroxidase activities of the treated plants increased appreciably compared to untreated plants and declined by re-watering. The results indicated that the stimulated activities is well correlated with the decrease of $CaCl_2$ (Table 7a-b). These results Indicate that in highly stressed plants, the treatment with $CaCl_2$ can stimulate the synthesis and activities of antioxidant systems. (Cheruth et al, 2007 & Parvaiz et al, 2014) recorded an increased production of mRNA for ascorbic peroxidase and superoxidase dismutase (SOD) and enhanced enzymatic activities of these proteins in *vigna membranacea* plants subjected to drought stress. The changes in sugars, amino acids and proline contents in $CaCl_2$ – treated *vigna membranacea* plants was accompanied with a progressive increase in carbohydrate and protein contents indicating a promotion of growth in the treated plants, this enhancement of growth may be related to the increased flux of water and to the decrease in potential difference between plants and soil. In other words, any of these $CaCl_2$ concentrations can alleviate, to some extent, the adverse effects of drought stress on plant metabolism, storage functions and defense, this inclusion is agreement with the previous view of Naeem et al, 2013).

Table (6):): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on seedlings proline accumulation (mg \g F.w.)

Treatment $CaCl_2$.CO.	Before water stress	During water stress				after four hours of water stress			
	100%	100%	75%	45%	35%	100%	75%	45%	35%
0	41.22	53.00	45.81	54.45	68.45	62.34	55.23	84.48	82.95
10^{-4}	88.04	73.79	97.00	99.62 *	96.95*	80.91	38.28	70.48	73.03
10^{-3}	90.08	73.28	84.99	89.57*	126.21*	82.06	73.42	58.27	86.00
10^{-2}	87.03	73.67	109.67	117.56*	119.59*	49.62	103.56	38.42	98.22

Table (7): The impact of soaked seeds of *Vigna membranacea* in different calcium chloride concentration on the activity of enzymes in the leaves of seedling (a. amylase)

Treatment $CaCl_2$.CO.	before water stress	During water stress				after four hours of water stress			
	100%	100%	75%	45%	35%	100%	75%	45%	35%
0	2.16	3.41	4.78	4.43	3.70	3.23	6.24	5.44	5.92
10^{-4}	2.02	4.02	14.44**	13.65**	10.65**	4.10	6.79	5.03	2.60
10^{-3}	3.37	5.47	6.67**	10.44**	10.12**	5.60	4.59	3.05	4.20
10^{-2}	2.31	3.70	5.81	4.49	3.16	2.03*	0.93**	1.33*	3.80**

B: peroxidase

Treatment $CaCl_2$.CO.	before water stress	During water stress				after four hours of water stress			
	100%	100%	75%	45%	35%	100%	75%	45%	35%
0	4.81	7.44	11.28	11.46	11.63	6.19	14.35	10.77	11.73
10^{-4}	9.59	12.07	11.07	10.53	10.31	12.29*	11.22	12.03	18.92
10^{-3}	5.55	11.97	8.66	8.17	14.77	12.17*	15.48	6.95	11.59
10^{-2}	3.87	11.36	6.40	8.88	16.98	11.82*	4.08	11.53	12.17

Authors:
H. M. Mandururah & A. A. Alayafi
Department of Biological Sciences, Girls Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
Corresponding Author: aal_shareaf@hotmail.com

REFERENCES

1. Bates. L.S; Waldran. R.P. and Teare. L.D. (1977): Rapid determination of free proline for water stress studies. Plant and Soil 39, 205-208.
2. Chengbin. X; Xuemei. L; Lihong. Z. (2013): The Efffect of Calcium Chloride on Growth, Photosynthesis, and Antioxidant Responeses of *Zaysia Japonica* under Drought Conditions. Journal. pone. 0068214. Dol:10.1371.
3. Cheruth.A; Paramasivam. M; Sankar. B; Ashok. K; Rajaram. P.(2007): Calcium chloride effects on salinity –induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. Comptes Rendus Biologies, Vol.330(9):674-683.
4. Das.G. and Sen-Mandi. S. (1992): Scutellar amylase activity in naturally aged and accelerated aged wheat seeds. Annals of Botany 69,497-501.
5. Dubey. R.S. and Sharma. K.N. (1990): Behaviour of phosphatases in germinating rice in relation to salt tolerance. Plant Physiology and Biochemistry.Vol. 28(1):17-26.
6. Farooq. M; Wahid. A; Kobayashi.N; Fujite. D; Basra. S.M.A. (2009): Plant Drought Stress: Effects, Mechanisms and Management. Sustainable Agriculture. PP.153-188.
7. Hadas, A. (1976):- Water uptake and germination of leguminous seeds under changing external water potential in osmotic solution. J. Exp. Bot. 27,480-489.
8. Harrak. H; Chamberland. H; Plante. M; Bellemare. G; Lafontaine. J. G. & Tabaeizadeh. Z. (1999): A proline, Threonine, and Glycine- Rich Protein Down- Regulated by Drought is localized in the Cell Wall of Xylem Elements. Plant Physiol. 121(2) 557 - 564.
9. Hassanein. R.A; Bassuony. F.M; Baraka. D.M; Khalil. R.R (2009): Physiological Effects of Nicotinamide and Ascrobic Acid on *Zea mays* Plant Grown Under Salinity Stress. Journal of Agriculture and Biological Sciences, 5(1):72-81.
10. Hatree.E.F.(1972): A modification of the lowery method that gives a linear photometric response. Anal. Biochem. 48,422-425.
11. Kar. M.and Mishra. D. (1976): Catalase, peroxidase and polyphenol oxidase during rice leaf senescence. Plant Physiol. 57,315-319.
12. Kim J. Y; Mahe.A; brangeon. J. & Prioul.J. L. (2000): A maize vacuolar invertase,1VR2, in induced by water stress. Organ / tissue specificity and diurnal modulation of expression. Plant Physiol. 124, 71 - 84.
13. Lichtenthaler. H.K., (1987): Chlorophylls and carotenoid: pigment of photosynthetic biomembranes. Methods Enzymol. 184,350-382.

14.Marjetka. K; Janko.K; Jerica. S.(2014): Proteases and their endogenous inhibitors in the plant response to abiotic stress. Botanica SERBICA, 38(1):139-158.

15.Marie. L.Z; Osbaldo. R (2013): A Roadmap Towards a Systems Biology Description of Bacterial Nitrogen Fixation. Soil Biology. Vol.37, pp 27-51.

16.Michelle. M, Mohammad. B, Sheng-Chun. X, Andrew. O, Xiao-Hui. L, Ya Ming. G, Paul. H, Zhong- Hua. C. (2014): Leaf mesophyll K^+,$H^+$and Ca^{2+} fluxes are involved in drought – induced decease in photosynthesis and stomatal closure in soybean. Environmental and Experimental Botany. Vol.98:1- 12.

17.Naeem.M; Khan. M.N; Masroor.M; Moinuddin. (2013): Adverse Effects of Abiotic Stresses on Medicinal and Aromatic Plants and Their Alleviation by Calcium. Plant Acclimation to Environmental Stress. pp 101-146.

18.Naguib. M.I.(1964): Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds. Ind.J.Exp.Biol.2,149-152.

19.Naguib.M.I.(1969): On the colorimetry of nitrogen compounds of plant tissue. Bull.Fac.Sci. Cairo University. 43,1-5.

20.Parvaiz.A;Asiya.H;Elsayed.F.A;Subzar.A.S;Mohd. R. W; Saiema.R; Sumiya. J; Ashwani.K.(2014): Biochemical and Molecular Approaches for Drought Tolerance in Plants. Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment.pp:1-29.

21.Pinheiro.C and Chaves. M.M. (2011): Photosynthesis and drought: can we make metabolic connections from available data ? J. Exp. Bot.62(3): 869-882.

22.Pelah.D;Wang.W;Altman.A; Shoseyov. O; Bartels. D. (1997):-Differential accumulation of water stress- related proteins,sucrose synthase and soluble sugars in *populus* species that differ in their water stress response. Physiol. Plant, 99,153-159.

23.Ron.M and Eduardo. B. (2010): Genetic Engineering for modern Agriculture: Challenges and Perspectives. Plant Biology.Vol.61:443-462.

24.Rosa. M.R; Teresa. C.M; Ron. M; Francisco. R; Francisco. G; Vicente. M. (2014):The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant,Cell & Environment. Vol.37(5)1059-1073.

25.Shi-Lin.T; Bo -Ya.L; Zhen-Hui. G; Syed. N. M. S. (2014): Effects of drought stress on capsanthin during fruit development and ripening in pepper (*Capsicum annuum* L.) Agricultural Water Management. Vol.137:46-51.

26.Santiago.S; Francisco.J.C; Omar.B,Juan.B.B, Jorge. M.(2013): Water stress induces a differential and spatially distributed nitro-oxidative stress response in roots and leaves of *Lotus Japonicus*. Plant Science.Vol.201:137-146.

27.Thangella.A.V.P; Daggu.M.R.(2013): Differential accumulation of osmolytes in 4cultivars of peanut (*Arachis hypogaea* L.) under drought stress. Journal of Crop Science and Biotechnology. Vol.16(2):151-159.

28.Vaz.M;Pereira.J.S;Gazarini.L.C;David.T.S;Rodrigues. A; Maroco.j; Chaves.M.M. (2010):Drought –induced photosynthetic inhibition and autumn recovery in two Mediterranean oak species *(Quercus ilex and Quercus suber).*Tree Physiology 30(8):946-956.

29.Wim.V; Edoardo. B; Stefanie. D.B; Klaas. V; Marlies. D; Mario.E.P; Dirk. I. (2013): Molecular and Physiological Analysis of Growth Limiting Drought Stress in *Brachypodium distachyon* leaves. Mol. Plant 6(2): 311-322.

1/15/2015