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Research Article
Signal Molecules Improving Growth, Yield and Biochemical Aspects of Wheat Cultivars under Water Stress

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Abstract

Background and Objective: Using signal molecules as calcium (Ca^{2+}) and nitrogen oxide (NO) have an important role on alleviation of the adverse effects of drought stress on different plant crops. **Materials and Methods:** Thus, a field experiment was carried out during two winter successive seasons to study the beneficial role of calcium chloride (Ca source) and sodium nitroprusside (SNP as No donor) with different concentrations of two cultivars of wheat plant (Seds 13 and Sakha 94) under drought stress. **Results:** Growing wheat plants under different levels of water irrigation requirement (WIR) (75 and 50%) caused significant gradual decreases in different growth criteria, photosynthetic pigments and yield components as compared with control plants (those plants irrigated with 100%). Meanwhile, increasing gradually total soluble sugars, proline and free amino acids, H_2O_2 and MDA contents compared with control plants. Soaking grains of the 2 cultivars (Seds 13 and Sakha 94) in $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) for 12 h before sowing improved growth and yield of wheat plant and could alleviate the reduced effect of drought stress on growth and yield of wheat plant through increasing photosynthetic pigments, total soluble sugars, proline and free amino acids contents. Meanwhile decreasing H_2O_2, MDA contents as compared with their corresponding controls. These treatments under water deficit led to appearance or disappearance of many bands were not present in the control depending on the wheat cultivars, evidence Sids 13 gave more bands than Sakha 94. **Conclusion:** In conclusion, $CaCl_2$ and SNP have a beneficial role in alleviating the toxic effect of drought stress on wheat plant.

Key words: $CaCl_2$, sodium nitroprusside, drought, wheat, H_2O_2, isozymes, protein pattern

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Egypt, one of the most important cereal crops is wheat (*Triticum aestivum* L.) and in the world, it is the third most-produced cereal after maize and rice, while it is the second to rice regarding to dietary intake[1]. Egypt suffers from drought problem which present in some arid and semi-arid regions. Thus, drought is considered as one of the primary factors responsible for crop productivity reduction[2]. Drought stress affect adversely on plant through decreasing growth, nutrient availability and alteration in water status of plants[3]. Drought reduced available water which led to decreases in photosynthetic efficiency via increased production and accumulation of reactive oxygen species[4]. Also decreased water content plant in cells lead to loss of cell enlargement and turgor in addition to the decrease in water potential of leaf. Metabolism disturbance, photosynthesis reduction and finally death of plant are the result of high water stress[5]. The respond of different plant to water stress varies significantly depending on the duration and intensity of stress as well as species of plant and the growth stage of plant[6].

In the last years and because of the great interest for both basic and applied research, there has been an important progress on understanding the mechanisms underlying abiotic stress adaptation and defense in different plant species[7]. Unfavourable abiotic or environmental conditions that faces various crops lead to evolution of different adaptive mechanisms by which plant cells can sense these environmental stimuli or signals and cause responses which help plant to tolerate and survive these adverse environmental conditions[8]. Sensing of biotic or abiotic stress conditions induces signaling cascades that activate production of signal molecules among them, reactive oxygen species (ROS), calcium (Ca^{2+}), nitric oxide (NO), accumulation of hormones such as abscisic acid, ethylene, jasmonic acid and salicylic acid. These signal molecules ultimately induce expression of specific subsets of defense genes that lead to the assembly of the overall defense reaction[9]. Plant responds to stresses either as individual cells or synergistically as a whole organism. Stress signals are first perceived by the receptors present on the membrane of the plant cells. Following this the signal information is transuded downstream resulting in the activation of various stress responsive genes.

Calcium is a universal signaling molecule and the calcium-sensing (CaS) receptor is of fundamental importance for extracellular calcium signaling and calcium homeostasis[10]. Calcium is an important second messenger in signal transduction pathways Achary *et al.*[11], mediating various defense responses to the action under environmental stresses[12]. Calcium (Ca^{2+}) is micro and multifunctional element in plants used in different biochemical and physiological processes[13]. Calcium is an essential nutrient for growth and development of plants which involved in various important functions as in stability of plant membrane and stabilization of cell wall as well as, increases a huge number of key enzymes activities and interacting with phytohormones[14]. Moreover, it serves in the signaling network pathways as a secondary messenger under different abiotic stress[15]. In addition, calcium appears to play a central role in many defence mechanisms which are induced by stress and calcium signaling is required for the acquisition of stress tolerance or resistance[16].

Nitric oxide (NO) another signal molecule is a small, highly diffusible gas and bioactive molecule. Its chemical properties make it a versatile signal molecule that functions through interactions with cellular targets via either redox or additive chemistry[17]. NO has attracted much attention because of its pivotal role in stress tolerance exerted by oxidative stress in various processes of plant growth, development, metabolism and cell death[18]. Neill *et al.*[17] mentioned that, NO play an important role in cellular protection against toxicity of ROS, defense response and tolerance to abiotic stress. Moreover, NO can mediate plant growth regulators and ROS metabolism as well as, NO involves in signal transduction and responses to biotic and abiotic stresses[19]. Uchida *et al.*[20] reported that tolerance to drought, salt and heat stresses was enhanced in tomato, wheat and rice seedlings when the plants were treated with NO donor.

So, the target of our study was to study the effect signal molecules (Ca^{2}+and NO) on growth, some physiological and biochemical aspects, yield quantity and quality of 2 cultivars of wheat plant grown in reclaimed sandy soil conditions by using the different amount of water irrigation requirements (WIR).

MATERIALS AND METHODS

A field experiment was carried out at the experimental Station of National Research Centre, (latitude 30° 30' 1.4' 'N, longitude 30°19' 10.9'' E and mean altitude 21 m above sea level) Nubaria district El-Beheira Governorate-Egypt, (North Africa represent arid or semi-arid region) during two winter season of 2016/2017 and 2017/2018. Grains of wheat cultivars (Sakha 94 and Seds 13) were obtained from the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Calcium chloride or Sodium nitroprusside used in the present work were supplied from Sigma-Aldrich. The soil of experimental

Table 1: Mechanical, chemical and nutritional analysis of the experimental soil

Seasons	Constant depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture class
2017	00-30	40.7	44.6	10.7	4	Sandy
	30-60	38.2	43.0	13.8	5	Sandy
2018	00-30	38.7	42.6	13.7	5	Sandy
	30-60	36.5	38.1	17.8	7.6	Sandy

	Constant depth (cm)	pH	Electrical conductivity (dS m^{-1})	Saturation (%)	Anions (milliequivalents/liter) CO_3^{-2}	HCO_3^-	Cl	SO_4^{-2}	Cations (milliequivalents/liter) Ca^{++}	Mg^{++}	Na^+	K^+	$CaCo_3$ (%)	Organic mater (%)
2017	00-30	7.84	1.17	32	-	0.50	8.40	1.11	1.80	0.90	7.10	0.20	1.00	0.40
	30-60	7.89	1.79	27	-	0.60	8.00	1.40	2.10	1.50	6.20	0.20	6.00	0.07
2018	00-30	7.95	1.59	23	-	0.32	12.70	1.98	4.00	1.80	9.00	0.20	1.90	0.38
	30-60	7.85	1.81	25	-	0.45	15.40	2.15	5.60	2.00	10.20	0.20	1.30	0.32

site was reclaimed sandy soil where mechanical and chemical analysis is reported in (Table 1) in Research Approach and Methodology according[21].

Wheat grains were soaked in different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) for 12 h before sowing. The experimental design was split-split plot the amount of water irrigation requirements (WIR) 100, 75 and 50% occupy the main plots, while the two wheat cultivars (Sids 13 and Sakha 94) were randomly assigned in sub plots and the treatments of ($CaCl_2$ and NO) were allocated at random in sub-sub plots. Grains of wheat were sown at November 25th in rows 3.5 m long and the distance between rows was 20 cm apart, Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in Length). The recommended agricultural practices of growing wheat grains were applied and the seeding rate was (144 kg grains ha^{-1}). Pre-sowing, 360 kg ha^{-1} of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 180 kg ha^{-1} was applied at 5 equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added at 2 equal doses of 120 kg ha^{-1}, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days according to the amount of water used.

Plant samples were taken after 75 days from sowing for measuring growth characters in terms of plant height (cm), leaves no/tiller, tiller and root fresh and dry weights (g). Samples of plant were taken for biochemical analysis as photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), TSS, proline, free amino acids, protein electrophoretic pattern and antioxidant enzymes. At harvest the following characters were recorded on random samples of 10 girded plants in each plot to estimate the following characters: Plant height (cm), spikelet no/spike, 1000 grains weight (g), grains yield/spike (g), straw yield (t ha^{-1}), biological yield (t ha^{-1}), grain yield (t ha^{-1}) an d the nutritional values of grains yield.

Irrigation water requirements: Three irrigation water requirements was calculated using Penman Monteith equation and crop coefficient according to Allen *et al.*[22]. The average amount of irrigation water applied with sprinkler irrigation system were 5950, 4462.5 and 2975 m^3 ha^{-1} season-1 as (100 and 75%, respectively) for both seasons of 2016/2017 and 2017/2018.

The amounts of irrigation water were calculated according to the following equation:

$$IWR = \frac{ET_0 \times Kc \times Kr \times I + LR}{Ea} \times 4.2$$

Where:

IWR = Irrigation water requirement m^3/ha/irrigation
ET_0 = Reference evapotranspiration (mm/day)
Kc = Crop coefficient
Kr = Reduction factor[23]
I = Irrigation interval, day
Ea = Irrigation efficiency, 90%
LR = Leaching requirement = 10% of the total water amount delivered to the treatment

Water-use efficiency (WUE): WUE values calculated with the following Eq.[24]:

$$WUE = \frac{E_y}{E_t} \times 100$$

where, WUE is the water use efficiency (kg m^{-3}), E_y is the economical yield (kg ha^{-1}. season), E_t is the total Applied of irrigation water, m^3 ha^{-1}/season.

Chemical analysis: Photosynthetic pigments: Chlorophyll a, chlorophyll b and carotenoids were determined using spectrophotometric method described by Lichtenthaler and Buschmann[25]. Total soluble sugars were extracted by the

method of Prud'homme *et al.*[26] and analyzed according to Yemm and Willis[27]. Free amino acids and proline were extracted according to the method described by Vartainan *et al.* [28]. Free amino acid was determined with the ninhydrin reagent method[29]. Proline was assayed according to the method described by Bates *et al.*[30]. Total carbohydrate was determined according to DuBois *et al.*[31]. The level of lipid peroxidation was measured by determining the levels of malondialdehyde (MDA) content using the method of Hodges *et al.*[32]. Hydrogen peroxide (H_2O_2) content was determined using the method of Velikova *et al.*[33].

Electrophoretic analysis of protein by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was done according to Laemmli [34] as modified by Studier[35]. For the assay of antioxidant enzymes peroxidase (POX) and polyphenol oxidase (PPO) were extracted based on the method described by Stagemann *et al.*[36]. PPO and POX isozymes were separated by Native-polyacrylamide gel electrophoresis (Native-PAGE). The activities of POX and PPO were determined according to Brown [37] and Baaziz *et al.*[38].

Grain protein content (%) and grain starch content (%) were determined using the non-destructive grain analyzer, Model Infratec TM 1241 Grain Analyzer, ISW 5.00 valid from S/N 12414500, 1002 5017/Rev.1, manufactured by Foss Analytical AB, Hoganas, Sweden.

Statistical analysis: The obtained results were analysed on complete randomized design under split plot system analysis by M-STAT-C statistical analysis program[39]. Means were compared by using least significant difference (LSD) at 5%.

RESULTS

Growth parameters: Growth parameters of both wheat cultivars (Seds 13 and Sakha 94) plants in response to treatment with different concentrations of either $CaCl_2$ (20 and 40 mg L^{-1}) or SNP (1 and 2 mg L^{-1}) and grown under different water irrigation requirement (WIR) are presented in Table 2. Deceasing water irrigation requirement from 100-75 and 50% under the field conditions (drought stress) lead to marked, significant and gradual decreases in all growth parameter studied (plant height, number of leaves/tiller, tiller and root fresh and dry weight) except leaves number/tiller the decreases was non significant when compared to control plants grown under the level of 100% WIR of the two cultivars. On the other hand, treatment of the two cultivars Seds 13 and Sakha 94 of wheat plants with different concentrations of $CaCl_2$ and NO significantly increased all growth parameters under normal (100% WIR) and could partially alleviate the reduced effect of lower WIR 75 and 50% as compared with their corresponding untreated plants at different water levels as it increased significantly the tested parameters as compared with each corresponding controls except number of leaves/plant the increases were non significant (Table 2).

Photosynthetic pigments: Table 3 shows the influence of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of the two cultivars of wheat plants subjected to different levels of water irrigation requirement. Exposure of wheat plants to the 75 and 50% of WIR led to a significant decrease in photosynthetic pigments when compared to plants grown under the level of 100% WIR (Table 3). Treatment of wheat plants with different concentrations of $CaCl_2$ and NO increased significantly photosynthetic pigments under different water levels as compared with their corresponding WIR. The maximum increases in total pigments were obtained by using SNP (2 mg L^{-1}) and using $CaCl_2$ (40 mg L^{-1}) at 100, 75 and 50% WIR as compared with their corresponding WIR% in both cultivars.

Compatible solutes: Total soluble sugars (TSS), proline and free amino acids (FAA) contents of the two cultivars of wheat plant was significantly increased by decreasing water irrigation requirements from 80-75 and 50% relative to control plants (100% WIR) (Table 4). Meanwhile, all applied treatments either $CaCl_2$ or SNP with different concentrations significantly increased the above mentioned parameters in unstressed plants or drought stressed plants as compared with their corresponding untreated controls. Data presented in Table 4 show that, higher concentrations of either $CaCl_2$ (40 mg L^{-1}) or NO (2 mg L^{-1}) was more effective than lower concentrations (20 mg $CaCl_2$) or (1 mg L^{-1} NO).

Lipid peroxidation and H_2O_2 content: Lipid peroxidation expressed by malondialdehyde (MDA) content and hydrogen peroxide (H_2O_2) contents clearly showed significantly increases under different drought stress (75 and 50% WIR) as compared with their corresponding untreated controls in the 2 tested wheat cultivars Seds 13 and Sakha 94 (Table 5). These increases were gradually increasing with increasing drought levels. Meanwhile, MDA and H_2O_2 contents were significantly decreases in the both wheat cultivars treated with different concentrations of $CaCl_2$ or NO as compared with their corresponding untreated controls.

Table 2: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) on morphological criteria in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing (combined analysis of 2 seasons)

Cultivars	WIR (%)	Treatments (mg L^{-1})	Plant height (cm)	Leaves (no/tiller)	Tiller fresh wt (g)	Tiller dry wt (g)	Root fresh wt (g)	Root dry wt (g)
Seds 13	100	Control	47.35±0.33	5.67±0.33	3.37±0.06	0.91±0.01	2.04±0.10	1.53±0.09
		$CaCl_2$ 1	55.00±0.58	6.00±0.00	4.02±0.50	1.01±±0.04	2.16±0.16	1.56±0.07
		$CaCl_2$ 2	59.00±1.20	6.33±0.33	5.33±0.08	1.26±0.01	3.44±0.08	1.88±0.10
		NO 1	52.00±0.58	6.67±0.00	4.91±0.05	1.19±0.01	2.73±0.06	1.44±0.06
		NO 2	51.00±1.15	6.33±0.33	5.13±0.02	1.26±0.07	2.87±0.08	1.35±0.02
	75	Control	43.33±1.45	5.33±0.33	3.11±0.05	0.83±0.03	1.67±0.05	0.53±0.04
		$CaCl_2$ 1	47.67±1.53	6.33±0.33	3.82±0.13	1.02±0.04	1.88±0.01	1.05±0.05
		$CaCl_2$ 2	48.00±1.20	7.00±0.00	3.95±0.05	1.14±0.02	1.68±0.02	0.80±0.01
		NO 1	43.00±1.00	6.33±0.33	3.35±0.08	0.98±±0.01	1.85±0.04	0.91±0.00
		NO 2	42.76±0.88	5.33±0.33	3.64±0.03	0.88±0.03	1.89±0.06	1.15±0.03
	50	Control	25.76±0.67	5.33±0.33	1.56±0.04	0.40±0.02	0.90±0.02	0.38±0.01
		$CaCl_2$ 1	29.99±0.00	6.00±0.58	1.76±0.01	0.55±0.02	1.36±0.01	0.46±0.00
		$CaCl_2$ 2	30.62±1.00	6.00±0.00	2.23±0.05	0.49±0.00	1.05±0.02	0.34±0.02
		NO 1	34.39±0.88	6.33±0.33	2.70±0.10	0.64±0.01	1.09±0.04	0.42±0.01
		NO 2	35.80±0.88	6.00±0.00	2.78±0.04	0.57±0.00	1.19±0.04	0.46±0.01
Sakha 94	100	Control	37.05±1.53	5.67±0.33	2.87±0.06	0.60±0.01	1.81±0.22	0.63±0.03
		$CaCl_2$ 1	40.33±0.33	5.67±0.33	3.99±0.04	0.98±0.02	2.37±0.32	1.39±0.10
		$CaCl_2$ 2	52.84±1.15	6.67±0.67	5.45±0.05	1.38±0.03	3.17±0.17	2.08±0.02
		NO 1	49.80±0.88	6.33±0.33	4.57±0.07	1.16±0.31	2.00±0.17	1.02±0.50
		NO 2	52.50±0.88	6.67±0.33	4.85±0.18	1.19±0.08	2.61±0.10	1.09±0.07
	75	Control	32.50±0.88	5.67±0.33	1.45±0.03	0.46±0.00	1.64±0.07	0.56±0.01
		$CaCl_2$ 1	37.50±0.00	6.33±0.33	2.48±0.04	0.50±0.02	1.41±0.18	0.82±0.12
		$CaCl_2$ 2	44.20±1.00	7.00±1.00	4.27±0.01	0.83±0.01	2.43±0.18	1.55±0.03
		NO 1	40.33±0.88	6.33±0.00	3.86±0.06	0.79±0.04	1.74±0.16	1.16±0.06
		NO 2	50.80±0.33	7.00±0.00	4.06±0.03	0.70±0.03	1.70±0.05	1.35±0.04
	50	Control	24.33±0.33	5.00±0.00	1.298±0.05	0.39±0.02	0.98±0.04	0.31±0.02
		$CaCl_2$ 1	32.50±1.00	5.33±0.33	1.79±0.003	0.56±0.03	0.91±0.04	0.44±0.02
		$CaCl_2$ 2	30.00±0.00	6.00±0.00	1.44±0.08	0.45±0.01	0.93±0.06	0.46±0.03
		NO 1	30.33±0.33	5.67±0.33	1.92±0.08	0.47±0.06	0.85±0.02	0.42±0.03
		NO 2	32.33±0.33	6.00±0.33	1.66±0.07	0.49±0.04	1.22±0.10	0.68±0.04
		LSD at 5%	2.64	ns	0.17	0.18	0.03	0.02

$CaCl_2$1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1 mg L^{-1}, NO 2: 2 mg L^{-1}, ns: Non-significant, each value represents the mean±standard error (n = 3)

Data presented in Table 5 show that, higher concentrations of either $CaCl_2$ (40 mg L^{-1}) or NO (2mg L^{-1}) was more effective than lower concentrations (20 mg $CaCl_2$) or (1 mg L^{-1} NO).

Protein banding patterns: The SDS-PAGE for total proteins of 18 treatments ($CaCl_2$ and NO) for wheat cultivars (Seds 13 and Sakha 94) with different water irrigation requirement (100, 75 and 50% WIR) are illustrated in (Fig. 1 and Table 6). A total number of twelve bands were detected with molecular weights (MWs) ranging from 72-8 kDa, whereas eight bands were polymorphic with 66.67% polymorphism. With regard to protein banding patterns, 4 common bands with MWs (72, 31, 24 and 18 kDa) with 33.3% monomorphic bands were recorded in all treatments. Seds 13 cultivar grew under water stress exhibit changes in the levels of protein bands in the three WIR the separation of (8, 9 and 8) bands but in Sakha cultivar there were no changes (7) bands.

The highest number of bands (10) bands was recorded in lane (3) 100% WIR+2 mg L^{-1} NO, Lane (8) 50% of WIR+20 mg L^{-1} $CaCl_2$ in Seds 13 and Lane (11) 100% of WIR+20 mg L^{-1} $CaCl_2$ in Sakha 94 (Fig. 1 and Table 6). One unique band was recorded in 100% of WIR (lane 10) and 100% WIR+20 mg L^{-1} $CaCl_2$ (Lane 11) in Sakha 94 cultivar with MWs (20 and 50 kDa), respectively. These bands could be considered as specific negative and positive markers. New proteins appeared in Seds 13 leaves at molecular weights 42 and 10 kDa (Kilo Dalton) in 75 and 50% WIR and 39 and 20 kDa in Sakha 94 as compared with control (at 100% WIR) were de-Novo synthesized in the plant grown under water stress. It is noticeable that treatment with different concentrations of $CaCl_2$ and NO under water deficit leads to appearance or disappearance of many bands were not present in the control depending on the wheat cultivars evidence Seds 13 gave more bands than Sakha 94. The protein bands at molecular weight 15 kDa in both cultivars can be considered as positive markers for $CaCl_2$ and it was noted that these bands

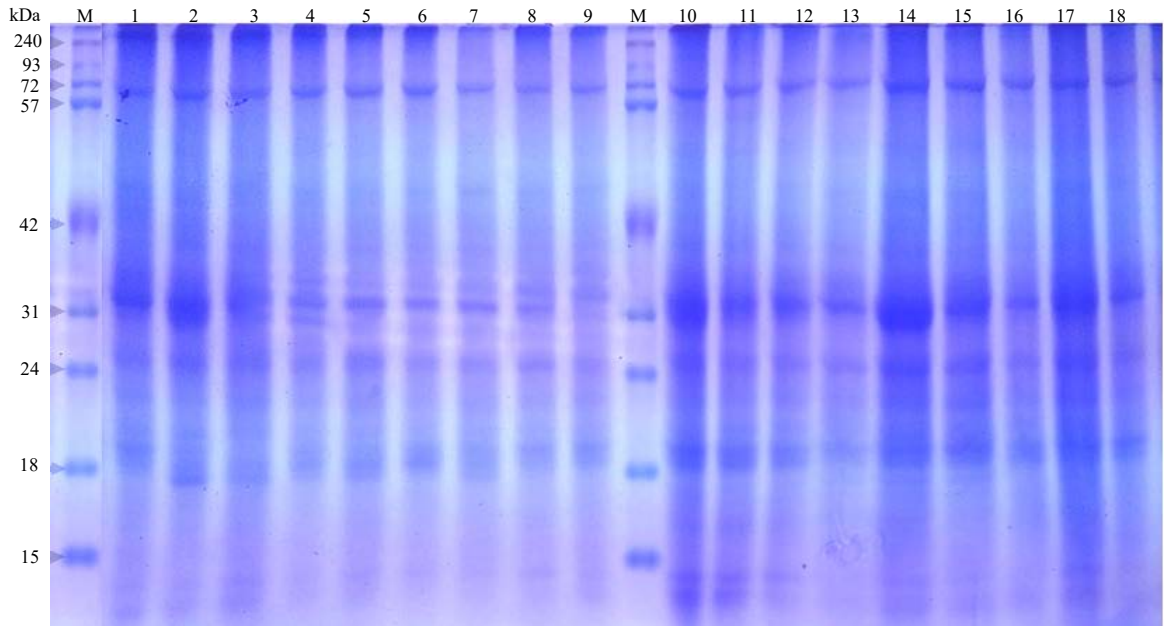

Fig. 1: Electrograph of soluble protein pattern by one dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) in response to different treatments ($CaCl_2$ and NO) on wheat cultivars with different water irrigation requirement (100 WIR, 75 WIR and 50% WIR). Seds 13 cultivar Sakha 94 cultivar

Each lane contains equal amounts of protein extracted from plant. Protein bands in the gel were visualized by Coomassie Blue Stain, Lane M: Marker, Seds 13 (Lane 1: 100% WIR, Lane 2: 100% WIR+20 mg L^{-1} $CaCl_2$, Lane 3: 100% WIR+2 mg L^{-1} NO, Lane 4: 75% WIR, Lane 5: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 6: 75% WIR+2 mg L^{-1} NO, Lane 7: 50% WIR, Lane 8: 50% WIR+20 mg L^{-1} $CaCl_2$ and Lane 9: 50% WIR+2 mg L^{-1} NO), Sakha 94 (Lane10: 100% WIR, Lane 11: 100% WIR+20 mg L^{-1} $CaCl_2$, Lane 12: 100% WIR+2 mg L^{-1} NO, Lane 13: 75% WIR, Lane 14: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 15: 75% WIR+2 mg L^{-1} NO, Lane 16: 50% WIR, Lane 17: 50% WIR+20 mg L^{-1} $CaCl_2$, Lane 18: 50% WIR+2 mg L^{-1} NO)

disapparent under the control and NO treatment at the three levels of WIR (%). Therefore, detection of proteins by SDS-PAGE revealed resistance for water deficit and induced some proteins directly or indirectly in cellular adaptations to stress.

Polyphenol oxidase (PPO): Isozyme patterns were investigated in 18 treatments of wheat seds 13 and Sakha 94 cultivars (Fig. 2 and Table 7). PPO-patterns displayed a total of 6 bands which are not necessarily present in all samples. Four of them show polymorphic (66.67%). The relative mobility (RF) of the bands ranged from 0.052-0.622. The other 2 with RF (0.052 and 0.378) were monomorphic bands at 2 cultivars. The largest number of bands was found in 50% WIR (Lane 7) (5 bands). It is noted that in 50% of WIR (Lane 7) gave a unique band with RF 0.622. This band could be considered as specific markers. It is noticeable that treatment with different concentrations of $CaCl_2$ and NO under water deficit is Seds 13 revealed some new bands combaring with Sakha 94 cultivar.

Peroxidase (POX): The isozyme banding patterns of the 18 treatments of wheat cultivars exhibited a maximum number of 8 bands at different Rf values varying from 0.201-0.905, whereas 7 bands were polymorphic with 87.5% polymorphism (Fig. 3 and Table 8). The other one with Rf 0.201 was monomorphic band from 2 cultivars and all treatments. The largest number of bands was found in (Lane 15) 75% of WIR+2 mg L^{-1} NO in Sakha 94 cultivar with high intensity. The minimum number of bands was found in the (Lane 10) 100% of WIR in Sakha 94 cultivar. Addition to, the band of 0.905 Rf which singly appeared in the (Lane 15) 75% of WIR+2 mg L^{-1} NO in Sakha 94 cultivar. Other recorded bands of the present investigation fluctuated between disappearance and appearance at different treatments with different intensity

Yield components: Table 9a, b shows the influence of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) on yield parameters of both cultivars of wheat plants subjected to different levels of water irrigation requirement. Exposure of plants to the 75 and 50% of water irrigation requirement (WIR) lead to a marked decrease in all yield parameters studied (spike length, spike weight, spikelet

Table 3: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) on photosynthetic pigments (mg g^{-1} fresh weight) in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

Cultivars	WIR (%)	Treatments (mg L^{-1})	Chlo a	Chlo b	Carot	Total pigments
Seds 13	100	Control	14.72±0.17	6.86±0.05	3.34±0.14	24.91±0.25
		$CaCl_2$ 1	17.58±0.15	5.04±0.18	6.17±0.09	28.79±0.41
		$CaCl_2$ 2	20.21±0.07	5.58±0.04	5.87±0.22	31.66±0.24
		NO 1	21.45±0.99	7.29±0.29	5.43±0.80	34.17±1.51
		NO 2	24.50±0.76	7.01±0.12	7.35±0.31	38.84±1.19
	75	Control	12.68±0.14	4.63±0.11	4.62±0.49	21.93±0.51
		$CaCl_2$1	15.95±0.07	5.42±0.48	4.82±0.48	26.19±0.08
		$CaCl_2$ 2	19.69±1.09	6.52±0.43	5.80±0.15	32.00±0.83
		NO 1	18.07±0.01	5.02±0.13	6.43±0.05	29.52±0.16
		NO 2	22.36±0.15	7.56±0.10	5.83±0.31	35.75±0.42
	50	Control	9.20±0.04	3.13±0.05	3.41±0.06	15.74±0.05
		$CaCl_2$ 1	14.51±0.01	4.54±0.20	4.68±0.05	23.73±0.16
		$CaCl_2$ 2	16.14±0.03	4.58±0.01	5.66±0.01	26.38±0.03
		NO 1	10.54±0.56	2.89±0.18	4.15±0.27	17.58±1.01
		NO 2	12.26±0.23	3.83±0.07	4.54±0.15	20.63±0.45
Sakha 94	100	Control	20.86±0.96	5.47±0.30	7.13±0.09	33.46±1.35
		$CaCl_2$ 1	22.34±0.11	5.71±0.16	7.67±0.22	35.72±0.04
		$CaCl_2$ 2	27.47±0.55	8.34±0.21	7.70±0.65	43.51±0.93
		NO 1	22.56±1.04	5.56±0.21	8.10±0.56	36.21±1.80
		NO 2	25.74±0.00	6.73±0.26	8.87±0.24	41.43±0.02
	75	Control	15.31±0.08	4.11±0.09	5.93±0.05	25.36±0.12
		$CaCl_2$ 1	19.11±0.05	5.42±0.27	6.17±0.46	30.70±0.25
		$CaCl_2$ 2	23.38±0.48	6.17±0.11	7.43±0.08	36.98±0.68
		NO 1	18.17±0.49	5.31±0.33	5.70±0.19	29.18±0.64
		NO 2	21.46±0.40	5.32±0.70	7.59±0.27	31.59±0.74
	50	Control	11.50±0.21	3.62±0.05	4.10±0.10	19.23±0.36
		$CaCl_2$ 1	15.31±0.08	4.11±0.09	5.98±0.03	25.40±0.14
		$CaCl_2$ 2	19.21±0.01	4.93±0.02	7.08±0.06	32.22±0.05
		NO 1	16.43±0.12	4.19±0.06	5.92±0.00	26.54±0.17
		NO 2	20.09±0.14	5.26±015	7.13±0.07	26.47±0.35
		LSD at 5%	1.51	0.69	0.87	2.02

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1mg L^{-1} NO 2: 2 mg L^{-1}

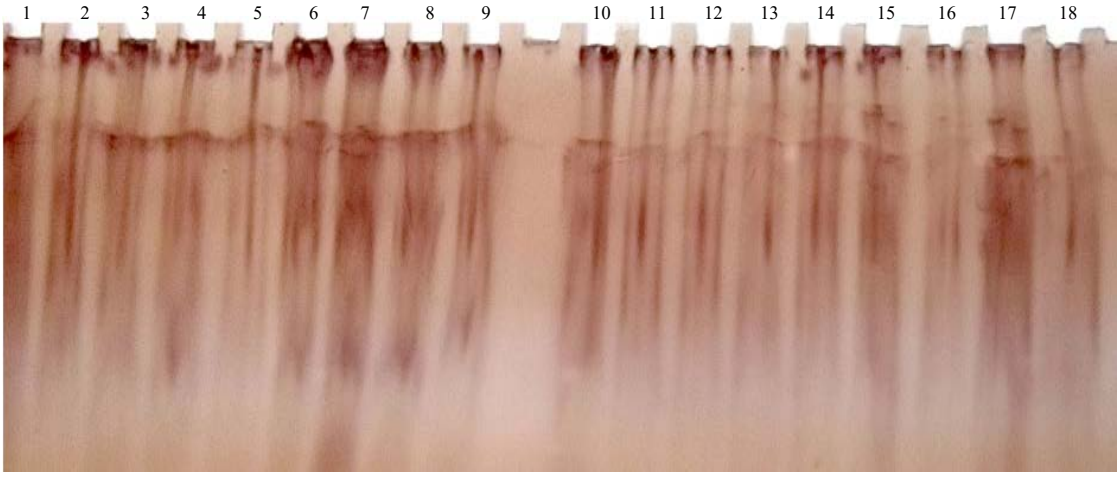

Fig. 2: Effect of $CaCl_2$ (20 mg L^{-1}) and NO (2 mg L^{-1}) on polyphenol oxidase (PPO) isozyme in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

Lane M: Marker, Seds 13 (Lane 1: 100% \WIR , Lane 2: 100% WIR+20 mg L^{-1} CaCl2, Lane 3: 100% WIR+2 mg L^{-1} NO, Lane 4: 75% WIR, Lane 5: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 6: 75% WIR+2 mg L^{-1} NO, Lane 7: 50% WIR, Lane 8: 50% WIR+20 mg L^{-1} $CaCl_2$ and Lane 9: 50% WIR+2 mg L^{-1} NO), Sakha 94 (Lane10: 100% WIR, Lane 11: 100% WIR+20 mg L^{-1} $CaCl_2$, Lane 12: 100% WIR+2 mg L^{-1} NO, Lane 13: 75% WIR, Lane 14: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 15: 75% WIR+2 mg L^{-1} NO, Lane16: 50% WIR, Lane 17: 50% WIR+20 mg L^{-1} $CaCl_2$, Lane 18: 50% WIR+2 mg L^{-1} NO)

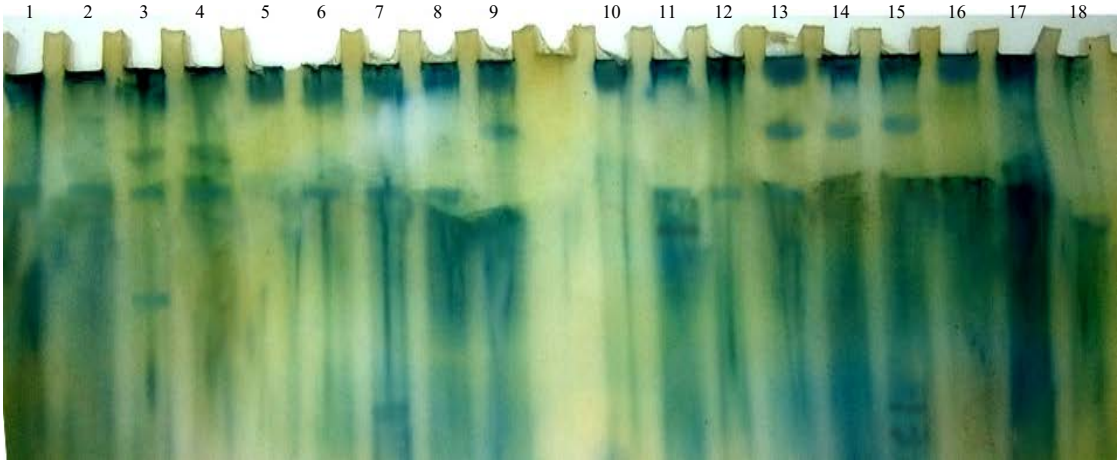

Fig. 3: Effect of $CaCl_2$ (20 mg L^{-1}) and NO (2 mg L^{-1}) on peroxidase (POX) isozyme in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

Lane M: Marker, Seds 13 (Lane 1: 100% WIR , Lane 2: 100% WIR+20 mg L^{-1} $CaCl_2$, Lane 3: 100% WIR+2 mg L^{-1} NO, Lane 4: 75% WIR, Lane 5: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 6: 75% WIR+2 mg L^{-1} NO, Lane 7: 50% WIR, Lane 8: 50% WIR+20 mg L^{-1} $CaCl_2$ and Lane 9: 50% WIR+2 mg L^{-1} NO), Sakha 94 (Lane10: 100% WIR, Lane 11: 100% WIR+20 mg L^{-1} $CaCl_2$, Lane 12: 100% WIR+2 mg L^{-1} NO, Lane 13: 75% WIR, Lane 14: 75% WIR+20 mg L^{-1} $CaCl_2$, Lane 15: 75% WIR+2 mg L^{-1} NO, Lane 16: 50% WIR, Lane 17: 50% WIR+20 mg L^{-1} $CaCl_2$ and Lane 18: 50% WIR+2 mg L^{-1} NO)

Table 4: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and SNP (1 and 2 mg L^{-1}) on total soluble sugars (TSS), proline and free amino acids (FAA) (mg/100 g) in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

Cultivars	WIR (%)	Treatment (mg L^{-1})	TSS	Proline	FAA
Seds 13	100	Control	2276±2.31	36.85±0.20	152.1±6.66
		$CaCl_2$ 1	2769±2.08	63.24±0.94	180.3±11.57
		$CaCl_2$ 2	3418±3.76	66.17±2.89	213.5±4.56
		NO 1	3155±3.53	54.07±0.43	200.5±12.21
		NO 2	3286±2.60	55.65±0.61	234.2±6.38
	75	Control	2830±7.86	47.29±0.01	366.9±6.70
		$CaCl_2$1	3418±10.39	78.79±0.95	441.2±1.70
		$CaCl_2$ 2	3544±11.55	73.05±1.18	386.1±1.33
		NO 1	3493±1.73	57.27±1.52	310.5±4.09
		NO 2	4143±5.77	62.05±1.10	412.8±1.39
	50	Control	3032±17.32	77.40±0.02	303.7±3.47
		$CaCl_2$ 1	3725±14.43	57.13±0.29	574.3±2.28
		$CaCl_2$ 2	3780±8.66	70.47±0.32	628.0±11.55
		NO 1	3583±17.13	67.97±1.20	611.5±5.20
		NO 2	3717±19.81	51.76±0.61	457.6±4.85
Sakha 94	100	Control	2463±13.86	33.17±3.74	147.0±0.87
		$CaCl_2$ 1	2544±14.53	37.38±0.76	160.7±0.05
		$CaCl_2$ 2	3502±13.86	45.37±0.08	192.0±1.07
		NO 1	3044±14.53	38.84±0.18	252.9±1.07
		NO 2	3221±25.40	64.25±0.28	215.9±5.83
	75	Control	3002±11.55	76.84±2.70	289.5±7.57
		$CaCl_2$1	3443±11.15	64.79±1.30	376.4±3.61
		$CaCl_2$ 2	3611±11.55	65.63±0.39	413.6±9.21
		NO 1	3339±16.35	81.84±0.27	378.6±2.60
		NO 2	3654±17.32	77.61±0.75	497.2±3.60
	50	Control	3334±28.87	87.36±0.18	560.5±5.54
		$CaCl_2$ 1	3791±15.77	78.21±1.03	564.0±2.60
		$CaCl_2$ 2	4292±14.62	89.05±1.19	590.0±0.00
		NO 1	3498±21.55	80.28±0.16	604.5±1.15
		NO 2	3785±17.32	83.56±0.05	620.9±1.21
		LSD at 5%	103.59	3.30	27.02

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1mg L^{-1}, NO 2: 2 mg L^{-1}

Table 5: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on H_2O_2 and MDA (μmol g^{-1} fresh wt) in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

Cultivars	WIR (%)	Treatments (mg L^{-1})	H_2O_2	MDA
Seds 13	100	Control	8.82±0.08	16.32±0.03
		$CaCl_2$ 1	8.22±0.06	14.96±0.04
		$CaCl_2$ 2	7.75±0.06	12.79±0.27
		NO 1	8.07±0.03	12.64±0.45
		NO 2	7.18±0.02	12.09±0.60
	75	Control	11.94±0.05	19.22±0.52
		$CaCl_2$1	9.28±0.04	17.91±0.09
		$CaCl_2$ 2	8.15±0.05	17.51±0.83
		NO 1	8.80±0.10	17.83±0.30
		NO 2	7.57±0.08	16.15±0.17
	50	Control	15.06±0.18	25.51±0.35
		$CaCl_2$ 1	11.63±0.06	20.91±0.04
		$CaCl_2$ 2	9.30±0.13	19.46±0.31
		NO 1	10.53±0.07	22.78±0.86
		NO 2	8.56±0.05	20.25±0.12
Sakha 94	100	Control	10.46±0.06	15.38±0.14
		$CaCl_2$ 1	9.97±0.05	13.58±0.43
		$CaCl_2$ 2	8.82±0.08	12.11±0.32
		NO 1	9.27±0.08	13.75±0.29
		NO 2	8.38±0.02	10.86±0.19
	75	Control	13.70±0.03	18.03±0.44
		$CaCl_2$1	11.82±0.10	15.85±0.42
		$CaCl_2$ 2	9.76±0.06	15.22±0.14
		NO 1	10.72±0.02	16.68±0.21
		NO 2	8.54±0.07	12.17±0.41
	50	Control	16.26±0.18	26.65±0.53
		$CaCl_2$ 1	13.43±0.13	23.98±0.44
		$CaCl_2$ 2	10.95±0.17	18.85±0.36
		NO 1	13.52±0.06	18.24±0.57
		NO 2	11.05±0.27	18.46±0.08
		LSD at 5%	0.29	1.22

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1 mg L^{-1}, NO 2: 2 mg L^{-1}

Table 6: Effect of $CaCl_2$ (20 mg L^{-1}) and NO (2 mg L^{-1}) on protein banding patterns of SDS-PAGE in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

		Seds 13									Sakha 94								
		100% WIR			75% WIR			50% WIR			100% WIR			75% WIR			50% WIR		
Band numbers	Mwt KDa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	72	15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	50											+							
3	42			+	+	+	+	+	+	+	+								
4	39	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
5	31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	20	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
8	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	17		+	+															
10	15					+			+			+	+		+			+	
11	10	+	+	+	+				+		+	+			+			+	
12	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Total No. of bands		8	9	10	9	9	8	8	10	8	7	10	8	7	9	7	7	9	6

+: Presence of band, Mwt: Molecular weight

Table 7: Effect of $CaCl_2$ (20 mg L^{-1}) and NO (2 mg L^{-1}) on polyphenol oxidase (PPO) isozyme in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

		Seds 13									Sakha 94								
Cultivars		100% WIR			75% WIR			50% WIR			100% WIR			75% WIR			50% WIR		
band number	Rf	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.052	++	+++	+++	+++	++	+++	+++	++	+++	+++	++	++	++	+++	+++	++	++	++
2	0.378	+	+	+++	+++	++	+++	+++	++	++	++	+	+	+	++	++	+	++	+
3	0.395										+					+		++	+
4	0.466		+	+	+		+	+	+	+	+							+	
5	0.583		+				+	+											
6	0.622							+											
Total number of bands		2	4	3	3	2	4	5	3	3	4	2	2	2	2	3	2	4	3

+: Faint, ++: Dark, +++: Very dark

Table 8: Effect of $CaCl_2$ (20 mg L^{-1}) and NO (2 mg L^{-1}) on peroxidase (POX) isozyme in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at 75 days from sowing

		Seds 13									Sakha 94								
Cultivars		100% WIR			75% WIR			50% WIR			100% WIR			75% WIR			50% WIR		
band number	Rf	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.201	+++	+	+	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	0.209									+				+	+	+			
3	0.218			+	+														
4	0.251	+	++	+	++	+	+	++	+	+		+	+	+	+	+	+	+	
5	0.313											++							+
6	0.488			+															+
7	0.829							+								+		+	
8	0.905															+			
Total number of bands		2	2	4	3	2	2	3	2	3	1	3	2	3	3	5	2	3	3

+: Faint, ++: Dark, +++: Very dark

number/spike, grain number/spike, grain weight/spike (g), weight of 1000 grain (g), straw yield, biological yield and grain yield t ha^{-1}), when compared to plants grown under the level of 100% WIR except the plant height in Sakha cultivars. Treatment of wheat plants with different concentrations of $CaCl_2$ and NO increased all yield parameter under different water levels as compared with the corresponding WIR%. The maximum increases in grains yield (t ha^{-1}) were obtained by using $CaCl_2$ (40 mg L^{-1}) followed by SNP (2mg L^{-1}) at 100, 75 and 50% WIR as compared with their corresponding WIR% in both cultivars. Meanwhile, Seds 13 surpassed the Sakha cultivars in grains yield (t ha^{-1}).

Carbohydrates, flavonoids and DPPH (%): Table 10 shows the influence of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on carbohydrates, flavonoids and DPPH percentages of both cultivars of wheat plants subjected to different levels of water holding capacity. Exposure of plants to the 75 and 50% of water capacity lead to a marked decrease in carbohydrates (%), when compared to plants grown under the level of 100% WIR, in both Seds and Sakha cultivars. Meanwhile, low WIR increased markedly flavonoids% and DPPH% of the 2 cultivars as compared with those plants irrigated with 100% WIR. Treatment of wheat plants with different concentrations of $CaCl_2$ and NO increased carbohydrates%, flavonoids% and DPPH% under different water levels as compared with the corresponding WIR (100, 75 and 50%) as compared with each corresponding untreated control plants.

Protein (%), glutine, zeleny and starch: The effect of foliar treatment of $CaCl_2$ or SNP on protein, glutine, zeleny and starch contents of the yielded grains of 2 cultivars of wheat plants grown under drought stress are presented in Table 11. Data clearly show that, irrigation of the 2 wheat cultivars with low irrigation water contents (WIR) 75 and 50% increased markedly protein, glutine and zeleny contents of the yielded wheat grains of Seds 13 and Sakha 94 cultivars. Meanwhile, 75 and 50% WIR decreased starch contents as compared with those plants irrigated with 100% WIR of both cultivars. Regarding to different $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}), $CaCl_2$ and NO caused more increases

Table 9a: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on yield components in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds13 and Sakha 94) (mean of two seasons)

Cultivars	WHC (%)	Treatments (mg L^{-1})	Shoot length (cm)	Tiller wt (g)	Spike length (cm)	Spikelet no/spike	Spike wt (g)	Grains wt/spike (g)	Grains no/spike	1000 grains wt (g)
Seds 13	100	Control	55.9±0.67	1.14±0.02	8.5±0.33	17.5±0.67	2.78±0.08	2.35±0.06	51.0±2.08	38.56±2.35
		$CaCl_2$ 1	60.0±3.18	1.40±0.06	11.3±0.29	19.5±0.33	2.98±0.02	2.46±0.02	54.5±0.88	43.24±0.48
		$CaCl_2$ 2	66.1±0.88	1.42±0.01	11.0±0.16	20.0±0.58	3.42±0.11	2.92±0.03	63.3±1.20	54.82±0.62
		NO 1	63.5±0.88	1.40±0.02	10.3±0.07	18.5±0.67	3.20±0.05	2.83±0.03	57.5±0.33	44.52±0.15
		NO 2	67.6±0.33	1.40±0.20	9.5±0.33	19.5±0.67	3.48±0.15	2.95±0.03	58.5±1.15	48.40±0.84
	75	Control	52.0±0.58	0.85±0.02	9.5±0.17	17.0±0.58	2.65±0.07	2.18±0.06	41.5±1.76	36.50±0.80
		$CaCl_2$ 1	61.5±0.31	1.16±0.19	10.5±0.00	19.0±0.58	2.84±0.07	2.24±0.01	44.5±1.76	40.50±0.90
		$CaCl_2$ 2	62.0±0.58	1.31±0.03	11.0±0.00	19.5±0.33	2.92±0.02	2.44±0.23	45.6±1.45	42.67±1.12
		NO 1	63.7±0.67	1.17±0.12	10.3±0.33	18.5±0.33	3.29±0.17	2.64±0.17	50.5±4.84	43.54±0.17
		NO 2	64.7±1.20	1.29±0.01	11.0±0.58	20.5±0.89	3.16±0.09	2.74±0.11	51.0±2.08	41.50±0.36
	50	Control	46.6±0.44	0.60±0.02	8.5±0.29	14.5±0.33	1.84±0.06	1.51±0.01	37.5±3.71	33.80±0.17
		$CaCl_2$ 1	57.1±0.17	0.96±0.02	10.0±0.58	16.0±0.58	2.44±0.15	1.84±0.00	40.2±0.33	35.63±0.41
		$CaCl_2$ 2	60.7±2.40	1.10±0.03	10.5±0.33	16.5±0.33	2.41±0.03	1.96±0.13	39.7±2.60	36.22±0.11
		NO 1	57.5±0.29	0.79±0.02	9.5±0.33	16.0±0.00	2.62±0.30	1.93±0.03	41.0±2.37	36.38±0.00
		NO 2	57.8±0.33	0.78±0.01	9.8±0.17	16.5±0.67	2.78±0.18	1.82±0.04	44.5±1.67	35.86±0.12
Sakha 94	100	Control	68.7±0.67	1.73±0.02	9.5±0.29	16.5±0.67	2.86±0.11	2.33±0.19	52.5±1.76	40.50±0.26
		$CaCl_2$ 1	71.0±0.58	1.87±0.08	10.5±0.29	17.0±0.00	2.92±0.23	2.45±0.26	54.7±1.20	42.55±0.36
		$CaCl_2$ 2	74.3±0.67	1.98±0.05	11.0±0.58	18.0±0.58	2.91±0.28	2.54±0.08	67.5±1.33	44.68±0.38
		NO 1	72.0±1.15	1.82±0.03	11.5±0.50	17.5±0.67	3.16±0.13	2.93±0.02	56.5±2.91	44.73±0.16
		NO 2	73.3±2.03	1.86±0.01	10.5±0.29	17.5±0.33	3.44±0.11	2.76±0.02	58.0±1.67	45.84±0.20
	75	Control	66.0±1.15	1.54±0.03	9.2±0.17	15.0±0.58	2.43±0.15	2.01±0.04	40.5±1.86	38.43±0.43
		$CaCl_2$1	73.6±2.96	1.73±0.03	10.2±0.17	17.0±0.58	2.95±0.12	2.41±0.24	44.0±1.15	41.53±0.29
		$CaCl_2$ 2	68.3±0.33	1.85±0.18	10.0±0.00	17.0±0.00	2.92±0.00	2.53±0.06	46.3±0.00	40.87±0.04
		NO 1	68.2±1.20	1.70±0.08	9.5±0.29	17.5±0.58	3.08±0.04	2.62±0.07	64.5±3.48	41.17±0.13
		NO 2	70.5±1.45	1.80±0.10	10.5±0.29	17.0±0.00	3.11±0.11	2.64±0.11	54.7±0.88	44.50±0.21
	50	Control	56.7±0.88	1.39±0.01	8.5±0.29	15.0±0.58	2.23±0.11	1.62±0.00	34.3±0.67	35.58±1.61
		$CaCl_2$ 1	62.0±0.58	1.52±0.09	10.5±0.29	17.0±0.58	2.75±0.07	2.00±0.00	39.5±2.08	39.42±0.00
		$CaCl_2$ 2	64.3±0.88	1.59±0.03	10.2±0.33	17.0±0.00	2.76±0.05	2.21±0.12	40.7±2.84	41.25±0.29
		NO 1	63.7±1.76	1.51±0.04	9.5±0.29	17.0±1.00	2.90±0.03	2.35±0.14	50.5±2.65	41.43±0.21
		NO 2	67.0±0.58	1.59±0.03	10.5±0.29	16.5±0.67	2.87±0.22	2.44±0.13	44.1.455±	42.92±0.33
		LSD at 5%	0.30	ns	1.00	ns	ns	0.21	ns	1.92

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1 mg L^{-1}, NO 2: 2 mg L^{-1}

in different protein constituents (Protein, glutine and zeleny%) and marked increases in starch contents as compared with their corresponding untreated controls either at normal irrigation (100%) or drought stressed (75 and 50%) conditions.

Water use efficiency (WUE): Table 12 shows the influence of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on water use efficiency of both cultivars of wheat plants subjected to different levels of water irrigation requirement. Exposure of plants to 75 and 50% of WIR led to a significant increase when compared to plants grown under the level of 100% WIR in both cultivars. Treatment of wheat plants with different concentrations of $CaCl_2$ and NO increased significantly water use efficiency under different water levels as compared with the corresponding control WIR% in both cultivars.

DISCUSSION

Growth parameters of both wheat cultivars were significantly decreased by decreasing WIR% Table 2. In agreement with the obtained results Alam *et al.*[40] showed that, water deficits affect plants in different ways, slowly developing water deficits decrease growth, by slowing rates of cell division and expansion due to loss of turgor which caused disturbances in water balance of stressed wheat plant leading to decreases in photosynthetic pigments (Table 3) and consequently retarded growth rate (Table 2). Furthermore, Khater *et al.*[41] stated that decreasing field capacity decreased different growth criteria of cowpea plant and they referred these decreases to disorders induced by stress and generation of reactive oxygen species (ROS).

Application of Ca^{2+} on wheat cultivars induced a significant increase in all tested growth parameters compared

Table 9b: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on yield components in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) (mean of 2 seasons)

Cultivars	WIR (%)	Treatments mg L^{-1}	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})
Seds 13	100	Control	7.15±0.05	11.83±0.01	4.68±0.02
		$CaCl_2$ 1	8.22±0.02	13.79±0.03	5.57±0.02
		$CaCl_2$ 2	8.24±0.01	14.35±0.02	6.11±0.02
		NO 1	7.46±0.06	13.26±0.06	5.80±0.01
		NO 2	8.37±0.14	14.00±0.13	5.63±0.13
	75	Control	7.02±0.15	11.15±0.17	4.13±0.02
		$CaCl_2$1	8.20±0.42	13.11±0.49	4.92±0.07
		$CaCl_2$ 2	8.24±0.28	13.72±0.31	5.48±0.03
		NO 1	9.22±0.28	14.14±0.31	4.92±0.03
		NO 2	9.29±0.07	14.39±0.03	5.09±014
	50	Control	6.07±0.05	9.30±0.04	3.02±0.00
		$CaCl_2$ 1	7.00±0.14	10.77±0.20	3.77±0.06
		$CaCl_2$ 2	5.93±0.17	9.75±0.32	3.82±0.15
		NO 1	5.44±0.15	9.24±0.25	3.80±0.09
		NO 2	6.00±0.14	9.95±0.19	3.95±0.04
Sakha 94	100	Control	6.54±0.14	11.28±0.16	4.74±0.02
		$CaCl_2$ 1	8.78±0.28	14.21±0.28	5.43±0.01
		$CaCl_2$ 2	8.78±0.00	14.59±0.08	5.81±0.08
		NO 1	10.07±0.18	15.86±0.04	5.79±0.14
		NO 2	9.37±0.18	15.08±0.14	5.72±0.04
	75	Control	7.66±0.20	11.69±0.20	4.03±0.00
		$CaCl_2$1	8.59±0.45	13.29±0.43	4.70±0.04
		$CaCl_2$ 2	9.10±0.09	14.40±0.18	5.30±0.11
		NO 1	7.88±0.32	12.67±0.31	4.80±0.02
		NO 2	9.56±0.28	14.41±0.31	4.85±0.07
	50	Control	5.78±0.52	8.82±0.59	3.01±0.04
		$CaCl_2$ 1	7.32±0.17	11.05±0.21	3.73±0.07
		$CaCl_2$ 2	8.17±0.14	11.95±0.12	3.78±0.03
		NO 1	7.85±0.31	11.57±0.34	3.72±0.09
		NO 2	7.63±0.17	11.38±0.16	3.75±0.18
		LSD at 5%	0.072	0.101	0.061

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1mg L^{-1}, NO 2: 2 mg L^{-1}

with control plants (Table2). Haleema *et al.*[42] confirmed the important role of Ca^{2+} in alleviating the adverse effect of abiotic stress on growth of tomato plant. The positive role of Ca^{2+} might be attributed to the fact that Ca^{2+} was suggested to be a crucial secondary messenger that used in signaling related processes to many defense mechanisms which are induced by stress[43]. Ca^{2+} can increase membrane stability, plays an important role in cell division, enlargement and protecting them from lipid peroxidation (Table 5) and oxidative stress induced by drought stress[44].

Regarding to sodium nitroprusside (SNP) (Table 2), the obtained results came on line with findings of Ramadan *et al.*[45] they stated that, pretreatment of sunflower plants with SNP increased the growth and yield under stress conditions. They attributed this effect to the NO capability to mitigate the adverse effects of stress on plants by improving leaf relative water content, photosynthetic pigment biosynthesis, osmolyte accumulation and antioxidative defense system. Moreover, exogenous treatment of sodium nitroprusside (NO donor) protected the two cultivars of wheat plants against drought stress-induced oxidative damage via promoting the biosynthesis of antioxidant enzymes (Table 7 and 8) and accordingly improving plant growth under drought stress.

Photosynthetic pigments contents significantly decreased by lowering water irrigation requirement (WIR). Meanwhile, treatment of wheat cultivars with $CaCl_2$ and SNP reduced the damages of lowering WIR on photosynthetic pigments (Table 3). These obtained data of decreasing photosynthetic pigments are congruent with those obtained earlier on quinoa[46]. Photosynthesis is the most sensitive process to drought stress[47], via reducing photosynthetic efficiency, stomatal conductance and inhibition in Rubisco activity[48]. Moreover, these reductions of photosynthesis might due to oxidation of pigments and impaired pigment biosynthesis[49].

The enhancement of photosynthetic pigment obtained in wheat leaves by foliar treatments of $CaCl_2$ had been proved previously on different species of wheat[50]. This promotive effect might be attributed to the effect of Ca^{2+} in preventing dehydration damage of cellular structures via maintaining the osmotic strength of cytoplasm in plants[51].

Table 10: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on carbohydrates%, flavonoids and DPPH% in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at grains yield (mean of two seasons). Each value represents the mean±standard error (n = 3)

Cultivars	WIR (%)	Treatments (mg L^{-1})	Carbohydrates (%)	Flavonoids (%)	DPPH (%)
Seds 13	100	Control	44.68±0.16	7.63±0.02	39.90±0.34
		$CaCl_2$ 1	46.47±0.12	13.08±0.00	22.24±0.14
		$CaCl_2$ 2	46.98±0.13	12.34±0.19	19.14±1.07
		NO 1	47.21±0.12	12.98±0.23	21.13±0.86
		NO 2	47.86±0.23	12.69±0.06	20.98±0.93
	75	Control	44.47±0.28	7.70±0.06	40.05±1.16
		$CaCl_2$1	44.68±0.19	17.78±0.09	37.83±0.12
		$CaCl_2$ 2	45.85±0.09	13.12±0.07	36.40±0.83
		NO 1	45.67±0.39	20.14±0.08	23.20±0.35
		NO 2	46.74±0.00	18.19±0.05	38.79±0.71
	50	Control	42.74±0.58	13.63±0.05	34.29±0.12
		$CaCl_2$ 1	43.74±0.29	15.47±0.06	21.13±0.07
		$CaCl_2$ 2	43.90±0.15	18.55±0.06	33.40±0.19
		NO 1	44.35±0.14	21.47±0.17	46.10±1.06
		NO 2	44.15±0.06	18.62±0.00	32.17±0.90
Sakha 94	100	Control	45.64±0.25	15.69±0.00	41.30±0.81
		$CaCl_2$ 1	47.85±0.12	18.57±0.01	41.40±1.01
		$CaCl_2$ 2	48.67±0.58	14.58±0.02	42.49±1.06
		NO 1	47.35±0.08	18.64±0.12	54.77±1.23
		NO 2	48.36±0.17	14.89±0.12	52.50±0.69
	75	Control	44.34±0.43	18.68±0.05	57.33±0.46
		$CaCl_2$1	44.95±0.25	20.15±0.12	59.01±0.39
		$CaCl_2$ 2	45.65±0.00	27.98±0.05	57.63±0.30
		NO 1	45.78±0.90	15.31±0.12	57.88±0.28
		NO 2	46.75±0.08	14.40±0.00	41.56±0.14
	50	Control	42.34±0.14	13.21±0.02	41.66±0.18
		$CaCl_2$ 1	43.52±0.18	19.46±0.08	69.89±0.12
		$CaCl_2$ 2	44.18±0.10	19.02±0.06	59.99±0.07
		NO 1	43.42±0.24	14.13±0.00	69.75±0.34
		NO 2	43.84±0.12	14.36±0.18	43.70±0.33
		LSD at 5%	ns	0.27	5.23

$CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1mg L^{-1}, NO 2: 2 mg L^{-1}

Regarding to NO effect, these obtained results are in agreements with those obtained earlier by Ramadan *et al.*[45]. This promotive effect can be explained by the potent role of NO in scavenging ROS and thus decreased oxidative damage in photosynthetic apparatus and increased chlorophyll content[52]. Moreover, Kausar, *et al.*[53] reported that NO was found to promote the improvement of photosynthetic pigments likely by defending the membrane of the cell organelle containing chlorophyll against stress-induced oxidative stress in chickpea plants.

Table 4 shows the promotive effect of drought stress on increased TSS, proline and free amino acids. This increment in TSS has also been found in shoot of different plant species subjecting to stress conditions[54]. TSS could act as ROS scavengers so improve membrane stabilization[55]. In addition, for adjusting cells and tissues water content, osmotic adjustment helps in turgor maintenance[47]. So, in case of water deficiency, plant cell will maintain water absorption and cell turgor, if the accumulation of osmolytes is sufficient to decrease cell osmotic potential[56]. With respect to proline and free amino acids, the hydrophilic character of proline gives it an important role in regulating osmotic potential[57]. In addition under a biotic stress, proline could act as a signalling molecule, modulate mitochondrial function and influence cell proliferation by triggering specific genes. An increase in free amino acid in drought stressed plants could be due to increased degradation of protein in higher plants[58].

Moreover, the improving effect of $CaCl_2$ on different osmolytes contents of wheat cultivars under both normal and stressed conditions might be related to its role in enhancing photosynthesis processes (Table 3). Regarding to SNP effect which increased proline content under drought stress, Ghadakchiasl *et al.*[59] confirmed this stimulating effect on *Rubus idaeus* plants under stress conditions. Lei *et al.*[52] reflected these increases to the increased activity of pyrroline-5-carboxylate synthetase (P5Cs) which is responsible of the synthesis of proline in wheat under drought stress.

Among different environmental stresses, drought stress was stated earlier to increase accumulation of ROS molecules[60] and these molecules could damage various cell constituents

Table 11: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on protein %, gluten, zeleny sedimentation index and starch in different levels of water irrigation requirement (WIR) of both wheat cultivars (Seds 13 and Sakha 94) at grains yield (mean of 2 seasons)

Cultivars	WIR (%)	Treatments	Protein (%)	Gluten (%)	Zeleny	Starch (%)
Seds 13	100	Control	12.00±0.21	29.5±1.32	40.8±3.14	67.3±2.32
		$CaCl_2$ 1	12.20±0.22	30.3±1.12	41.2±2.85	69.4±2.14
		$CaCl_2$ 2	13.50±0.24	33.2±1.08	45.5±1.67	69.1±2.25
		NO 1	12.50±0.25	30.4±0.98	42.9±1.74	68.9±2.47
		NO 2	13.20±0.31	31.1±1.04	42.4±1.67	68.9±1.96
	75	Control	12.80±0.32	33.4±1.32	44.0±2.84	64.0±3.01
		$CaCl_2$1	14.70±0.35	35.2±1.42	58.0±2.14	64.4±3.14
		$CaCl_2$ 2	15.70±0.34	37.4±1.24	58.0±2.64	66.4±2.85
		NO 1	12.60±0.31	31.6±1.34	50.3±1.84	68.4±2.69
		NO 2	15.20±0.09	33.5±1.25	50.7±2.34	66.7±2.35
	50	Control	13.60±0.07	33.5±2.31	53.2±2.74	63.5±2.85
		$CaCl_2$ 1	14.40±0.14	35.6±1.85	56.1±1.84	65.1±2.62
		$CaCl_2$ 2	14.00±0.11	36.0±1.95	56.1±2.14	65.4±2.74
		NO 1	13.00±0.13	34.4±2.31	56.2±2.07	67.6±2.34
		NO 2	13.80±0.14	34.2±3.14	59.3±2.06	66.5±2.74
Sakha 94	100	Control	12.40±0.08	32.2±2.41	45.2±0.00	65.6±2.15
		$CaCl_2$ 1	13.70±0.10	32.8±2.31	50.9±1.85	65.6±2.34
		$CaCl_2$ 2	12.90±0.08	33.4±2.74	46.0±1.65	67.5±1.95
		NO 1	13.60±0.75	33.2±1.34	48.6±1.85	66.0±1.74
		NO 2	12.70±0.36	33.4±1.74	48.9±2.64	66.4±1.34
	75	Control	13.20±0.41	34.9±2.34	46.5±2.84	64.5±2.35
		$CaCl_2$1	14.00±0.32	35.6±2.14	50.7±2.65	67.6±1.65
		$CaCl_2$ 2	14.10±0.41	36.0±2.74	52.8±1.52	65.6±0.95
		NO 1	13.60±0.15	35.6±3.14	53.0±1.85	65.8±0.78
		NO 2	13.60±0.34	36.3±2.74	50.8±1.27	66.8±1.02
	50	Control	13.60±0.15	35.6±2.04	58.6±1.62	63.5±1.32
		$CaCl_2$ 1	15.00±0.14	38.8±2.34	60.4±1.48	64.0±0.75
		$CaCl_2$ 2	15.20±0.34	39.2±2.74	62.4±1.62	65.1±1.62
		NO 1	15.70±0.41	40.5±2.64	66.6±1.52	64.0±2.01
		NO 2	14.90 ±0.32	38.5±2.34	63.8±2.14	64.8±1.35
		LSD 5%	0.284	1.032	3.425	3.954

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1 mg L^{-1}, NO 2: 2 mg L^{-1}

Table 12: Effect of different concentrations of $CaCl_2$ (20 and 40 mg L^{-1}) and NO (1 and 2 mg L^{-1}) on water use efficiency (Kg grain yield m^{-3}) of both wheat cultivars (Seds 13 and Sakha 94) subjected to different levels of irrigation requirement (WIR)

WIR (%)	Treatments (mg L^{-1})	Seds 13	Sakha 94
100	Control	0.79±0.012	0.80±0.052
	$CaCl_2$1	0.94±.0230	0.91±0.052
	$CaCl_2$ 2	1.03±0.017	0.98±0.046
	NO 1	0.97±0.040	0.97±0.040
	NO 2	0.95±0.029	0.96±0.092
75	Control	0.93±0.017	0.90±0.058
	$CaCl_2$1	1.10±0.087	1.05±0.029
	$CaCl_2$ 2	1.23±0.075	1.19±0.109
	NO 1	1.10±0.058	1.08±0.046
	NO 2	1.14±0.081	1.09±0.052
50	Control	1.02±0.069	1.01±0.006
	$CaCl_2$ 1	1.27±0.098	1.25±0.060
	$CaCl_2$ 2	1.28±0.081	1.27±0.060
	NO 1	1.28±0.104	1.25±0.040
	NO 2	1.33±0.091	1.26±0.087
	LSD 5%	0.03	

Each value represents the mean±standard error (n = 3), $CaCl_2$ 1: 20 mg L^{-1}, $CaCl_2$ 2: 40 mg L^{-1}, NO 1: 1mg L^{-1}, NO 2: 2 mg L^{-1}

as proteins, nucleic acids lipids and cell membranes[61]. H_2O_2 considered as the main ROS molecules. Lipid peroxidation, a non-enzymatic autoxidation process due to ROS, is commonly used as a measure of a biotic stress induced oxidative stress and plant sensitivity[60]. It is generally measured in terms of MDA contents, which are common end-products of lipid peroxidation. Our obtained results, showed significant increases for both lipid peroxidation and H_2O_2 contents

(Table 5). Hossain *et al*.[62] confirmed these obtained results, they stated that, drought stress increased MDA and H_2O_2 contents. These increment could be attributed to the inadequate induction of antioxidant system. Meanwhile, $CaCl_2$ and SNP decreased H_2O_2 and MDA contents, these results are similar to those obtained by Ramadan *et al*.[45] on different sunflower plant.

The changes in protein electrophoresis patterns extracted from the leaves of both wheat cultivars grown under different WIR% are shown in (Fig. 1 and Table 6). Several kinds of alterations are showed, some proteins were disappeared and other proteins were enhanced and synthesis of the new group of protein was produced. El-Bassiouny *et al*.[57] confirmed these obtained results in sunflower plant. New proteins appeared in Seds 13 leaves at molecular weights 42 and 10 kDa (Kilo Dalton) in 75 and 50% WIR and 39 and 20 kDa in Sakha 94 as compared with control (at 100% WIR) were de-Novo synthesized in the plant grown under water stress. It has been suggested that these proteins have an osmoprotectant function or protected cellular structures[63,64]. It is noticeable that treatment with different concentrations of $CaCl_2$ and NO under water deficit led to appearance or disappearance of many bands were not present in the control depending on the wheat cultivars evidence Seds 13 gave more bands than Sakha 94 (Fig. 1 and Table 6). Abdel Latef[65] found that, seed treatment with $CaCl_2$ led to the appearance of four polypeptides with Mwts (43, 27, 25 and 13 KDa). The protein bands at molecular weight 15 kDa in both cultivars can be considered as positive markers for $CaCl_2$ and it was noted that these bands disapparent under the control and NO treatment at the three levels of WIR%. In this connection, Shen *et al*.[66] found that $CaCl_2$ had a positive effect on total soluble protein levels in Rhododendrons plant.

The results were in conformity with Forde[67], who suggested that NO sensing must be considered as a possible role for one or more of seven NRT_2 proteins encoded in *Arabidopsis* genome.

The antioxidant enzymes content POX and PPO in the shoots of both wheat cultivars exposed to water stress were increased, (Table 7 and 8). These results were confirmed by the results of El-Bassiouny *et al*.[57] in sunflower plant under water stress. POX and PPO increases could be considered as an indicative of the increased production of ROS and a build-up of a protective mechanism to reduce oxidative damage triggered by stress experienced by plants[68]. The results showed that the activity of PPO and POX bands varied depending on cultivars and treatments under stress[64].

It is noticeable that treatment with different concentrations of $CaCl_2$ and NO under water deficit is in seds 13 revealed some new bands combaring with Sakha 94 cultivar (Table 7 and 8). Other recorded bands of the present investigation fluctuated between disappearance and appearance at different treatments with different intensity. In this connection Shen *et al*.[66] found that, CaCl2 had a positive effect on enzymatic antioxidant activity (POD and SOD). Isozyme analysis has several advantages as compared not only with morphological and physiological characters but also with other genetic markers, especially isozymes are mostly co-dominant with a simple Mendelian inheritance in most loci, so that the frequency of individual alleles is directly counted. Besides, isozymes can be resolved for most plant species regardless of habitat, size or longevity.

Deceasing water irrigation requirement in different growth stages affect plant yield and biochemical constituents of the plant and yielded seeds. These reductions in yield of wheat cultivars (Table 9a, b) might be resulted by decreases in growth criteria (Table 2) and photosynthetic pigments (Table 3). Meanwhile, external application of two wheat cultivars with different concentration of either $CaCl_2$ or NO under normal and drought stress conditions caused significant increases in all parameters of yield and its components as compared to the their corresponding control plants (Table 9a, b). Similar finding were reported earlier concurrent with our obtained results of $CaCl_2$ on different plant under abiotic stresses[42]. The positive role of Ca^{2+} might be attributed to that Ca^{2+} was suggested to be a crucial secondary messenger that used in signaling related processes to many defense mechanisms which are induced by salinity stress[43]. With respect to the promotive effect of sodium nitroprusside as NO donor on yield components of wheat plants Ramadan *et al*.[45], stated that NO treatment enhanced yield and yield components of sunflower plants.

The changes in carbohydrates, flavonoids content and antioxidant potentials of the yielded grains of wheat plant in response to $CaCl_2$ and sodium nitroprusside treatments under drought stress showed in (Table 10). Drought stress decreased carbohydrate contents, meanwhile increased flavonoids content and antioxidant activities. Sadak *et al*.[69] found that, total carbohydrate concentrations were decreased in faba bean plants under salinity stress. This decrease on carbohydrates contents is mainly due to the reduction in growth parameters (Table 2) and photosynthetic pigments (Table 3). Carbohydrate changes of the yielded grains are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration[69].

Flavonoids considered as plant secondary metabolites and have antioxidant activity *in vitro* and also act as

antioxidants *in vivo*[70]. The antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. The increased contents of flavonoid under drought conditions may reflect some kind of defence against stress conditions, since drought stress was accompanied by increased production of reactive oxygen species[70].

On the other hand, (Table 10) the stimulating effect of calcium chloride and sodium nitroprusside treatments on carbohydrate contents as well as flavonoids and total antioxidant activity of yielded grains might be due to the increases in growth parameters and photosynthetic pigments (Table 2 and 3). As well as, these increases in carbohydrate contents might be due to the increased photosynthetic output so increased carbohydrates formation in leaves and thus increased the translocation of carbohydrate from leaves to developing grains. Treatment of wheat plants with $CaCl_2$ or SNP resulted in a significant increase in flavonoids contents (Table 10). These observations reveal that the bioactive molecule of $CaCl_2$ or NO may be an inducer for the biosynthesis of secondary metabolites (flavonoids) which act as oxygen scavengers to reduce oxidative stress and, hence, increase the growth and yield wheat plant.

Data of Table 11 clearly show that, irrigation of the two wheat cultivars with low WIR (75 and 50%) increased significantly protein, glutine and xylene contents of the yielded wheat grains of Seds 13 and Sakha 94 cultivars. In this connection, Eckert, *et al.*[71] reported that, a high Zeleny value (sedimentation volume) is associated with a high protein content and good baking quality. Rezaei *et al.*[72] reported that, moderate water deficits increase grain protein content while a small decrease in grain yield. Meanwhile, WIR at 75 and 50% decreased starch contents as compared with those plants irrigated with 100% WIR of both cultivars. Emam, *et al.*[73] showed that, drought stress decreased starch contents of the grains of 2 rice cultivars. That, effect may be referred to the reduction in photosynthetic rate which interrupt the carbohydrate metabolism in leaves and might lead to the decrease in assimilate transported to the sink organs.

$CaCl_2$ and NO caused more increases in different protein constituents (Protein%, glutine% and xylene sedimentation) and marked increases in starch contents as compared with their corresponding untreated controls either at normal irrigation (100%) or drought stressed (75 and 50%) conditions (Table 11). The increments in the accumulation of starch contents of $CaCl_2$ and NO pretreated stressed wheat grains might be referred to the increases of photosynthetic pigments[74] thereby results in enhancement of carbohydrate synthesis. Moreover, Zhang, *et al.*[75] observed that, N application also had a significant increase on grain protein content.

Exposure of plants to 75 and 50% of WIR led to significant increase water use efficiency when compared to plants grown under the level of 100% WIR respectively in both cultivars (Table 12). In this connection, The WUE increased under water stress Abd El-Mageed *et al.*[76]. Treatment of wheat plants with different concentrations of $CaCl_2$ and NO increased water use efficiency under different water levels as compared with the corresponding control of WIR% in both cultivars (Table 12).

The increases in water irrigation requirement under different treatments indicates that the plant use different mechanisms in response to water deficit, such mechanism that enables it to reduce its water consumption while maintaining high biomass. In this connection, Jędrzejuk *et al.*[77] reported that, calcium alleviated the negative impact of water shortage on plants of *Salvia splendens* and *Ageratum houstonianum* by improving water used efficiency while growing these plants.

CONCLUSION

It could be concluded that drought stress adversely affected growth and biochemical parameters as compared with control plants. Application of $CaCl_2$ and NO significantly increased growth, yield quantity and quality via increasing photosynthetic pigments, total soluble sugars, proline and free amino acids contents. Meanwhile decreasing H_2O_2, MDA contents and the synthesis of new proteins. Moreover, wheat plant treated with $CaCl_2$ and NO gave higher technological characteristics of grain like carbohydrate%, starch%, protein%, glutine% and xylene sedimentation in grain yield of both cultivars. Seds 13 surpassed the Sakha cultivars in grains yield (t ha^{-1}).

SIGNIFICANCE STATEMENT

The results of the article are very good for implemented on a large scale in the new lands and the application of sustainable farming methods are safer to the environment. Such newly reclaimed sandy lands are known for their lack of water retention from the speed of evaporation due to high temperatures or filtration speed which suffers from problems hindering agriculture, which will help solve the problem of water deficiency and food gap. It can finally help in filling the food gap of grain crops in the third world.

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