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Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid

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Abstract: Two pot experiments were carried out in the screen of National Research Centre during two successive seasons 2012/13 – 2013/14 to clarify the role of citric acid or oxalic acid (0.0, 100 and 200 mg/l) in increasing the tolerance of wheat plants to late sowing conditions (high temperature stress during different stages of growth). Delaying date of sowing for one month induced a marked reduction in growth, produced poor grains quality (decrease in both carbohydrate and protein percentage) as a result of exposing wheat plants to natural high temperature during different developmental processes as compared with those sown at normal date. Late sowing plants exhibited marked decreases in endogenous promoters (i.e IAA, GA_3 and cytokinins) and nucleic acid contents (DNA & RNA) as compared to plants sowed at normal time, concomitantly with marked increases in ABA, malonaldhyde (MDA) and H_2O_2 contents. Activities of catalase and superoxide dismutase enzymes (CAT & SOD) were decreased significantly at late sowing date plants as compared with control normal sowed plants. Citric acid or oxalic acid treatment with different concentrations at the selected two dates showed marked increases in the studied growth parameters and improved grain nutritional values of the yielded grains, In addition, to increases in the amount of IAA, GA_3 and cytokinins, DNA and RNA with decreases in ABA contents, MDA and H_2O_2 contents compared with untreated plants. The activities of CAT and SOD enzymes increased significantly in response to different treatments of either citric acid or oxalic acid. The changes in protein electrophoretic pattern in wheat shoots at suitable and late sowing dates and treatment with citric acid or oxalic acid were investigated. Late sowing date induced the synthesis of new proteins, these proteins are members of heat shock proteins. Citric acid or oxalic acid induced synthesis of certain responsive proteins. These proteins might interfere with increasing thermotolerance of wheat plants
Key words: Antioxidant enzymes, Citric acid, DNA, Heat stress, Lipid peroxidation, Oxalic acid, Protein electrophoretic pattern, RNZ, Wheat.

Introduction

Wheat is one of the most important crops in Egypt; its optimum sowing date plays an important role in yield production. Planting wheat in its optimum sowing date would realize optimum season length and achieve high grain yield as a result of suitable weather conditions prevailing through different wheat growth stages[1]. In Egypt, wheat is grown mostly after harvesting of summer crops. Sowing wheat usually gets delayed beyond November due to late harvesting of rice, cotton or sugarcane etc. In such case, wheat growth and yield are adversely affected due to high temperature during growth and reproductive phases. The duration of grain filling in cereals (wheat) is determined principally by temperature[2]. In wheat, high temperature (< 31ºC) can decrease the rate of grain filling[3].

Late sowing reduced the duration of vegetative phase and decreased the dry matter accumulation in growth period[4]. Higher temperature stress either accelerates the formation of toxic reactive oxygen species (ROS), i.e., H_2O_2, OH^-, O^{-2} levels within plant tissues or impairs the normal defense mechanisms against ROS toxic effects. ROSs is highly reactive and toxic, and can lead to the oxidative destruction of cells. The consequences of ROSs formation depend on the intensity of stress and on the physicochemical conditions in the cell. It has been generally accepted that ROSs produced under stress are the decisive factor that causes lipid peroxidation, enzyme inactivation[5], etc. In normal conditions, lipid peroxidation is a natural metabolic process. Lipid peroxidation activation is one of the possible results of a rapid response to stress. One of lipid peroxidation products MDA was investigated in the present work. The content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress[6]. Moreover, ROS damage chloroplast, reduce carbohydrate synthesis and exportation, attack cell membranes, led to their degradation and leakage of cell solutes, denaturation of proteins and enzymes, damage of nucleic acids, degradation of chlorophyll and suppression of all metabolic processes, and finally senescence and death of cells and tissues[7].

Antioxidants play an important role in stress tolerance of crop plants which helps plants to ameliorate the bad effect of stress. Organic acids as antioxidants (such as citric acid and oxalic acid) play an important role in plant metabolism[8]. At environmental stress as heat stress, organic acids due to their molecules auto (ox-reduction) properties, act as cofactors for some specific enzymes, i.e., dismutases, catalases and peroxidases, those catalyzed breakdown of the toxic H_2O_2, OH, O^{-2} (radicals)[9]. Organic acids (citric acid or oxalic) as non enzymatic antioxidant in chelating these free radicals and protecting plant from injury could result in prolonging the shelf life of plant cells and improving growth characters[10]. Citrate is considered to be the most powerful organic anion, followed by oxalate and malate, to mobilize phosphorous in the soil[11].

The beneficial effect of this physical chemical action in the roots of wheat, buckwheat, legumes and triticale, can be interpreted by the formation of stable molecular complexes between carboxylic acids and metallic cations favoring the availability and absorption with increase in the vigor of the plant[12]. Organic acids have been implicated in various roles in the metabolic and physiological responses of plants to water stress[13]. The role of organic acids in water stress is well investigated by many authors[14&15] they reported that the synthesis and breakdown of organic acids serve as a mechanism for pH regulation in plant cell. Presoaked seeds of onion and maize with some organic acids (oxalic, citric and/or succinic) counteracted the suppressive effect of the relatively higher salinity levels[16].

The present investigation is directed to study the effect of foliar application of citric acid or oxalic acid on alleviating the harmful effects of late sowing treatment (warmer condition) on growth, yield quality. The changes in the endogenous phytohormones including; auxins, gibberellins, cytokinins and growth inhibitors (ABA) in shoots of wheat plants were also investigated. The nucleic acid levels, MDA, H_2O_2, SOD & CAT enzymes activities and protein electrophoratic pattern of wheat shoots were also studied.

2. Materials and Methods

The experimental plant used in this investigation was wheat (*Triticum durum*L.). Purestrain of grains obtained from Egyptian Ministry of Agriculture. The chemicals used in the present work were citric acid or oxalic acid. They were supplied from SIGMA – ALDRICH.This experiment was carried out to investigate theeffect of foliar spraying of citric acid or oxalic acid on alleviating the harmful effect of late sowing treatment (warmer condition) on wheat plants. This study was carried out on 18/11/2012 (normal date) and 18/12/2012 (late date) and repeated at 18/11/2013 and 18/12/2013. A homogenous lots of wheat grains *Triticum aestivum* var. Beni sweef were sown in pots (50 cm in diameter) containing equal amounts of clay and sandy soil by the ratio of 2:1. Fertilization was done with the recommended dose i.e (5 g phosphorous/pot as triple phosphate, 6 g nitrogen/pot as urea and5 g potassium/pot as potassium sulphate) during preparation of pots and after sowing. After 15 days from sowing thinning was carried out, so three uniform plants were left in each pot for studying the effect of different treatments on carbohydrate and protein contents of the yielded grains.The pots were divided into 5 groups each composed of 10 pots. The plants of each group were foliar sprayed with 0, 100 and 200 mg/l citric acid or oxalic acid, respectively. The treatments were carriedout twice after 60 and 75 days after sowing (DAS). The growth parameters of differently treated wheat plants were measured at 90 DAS.The studied growth parameters were plant height (cm), number of tillers/plant and dry weight of shoot.

The endogenous growth regulators, indole acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA), cytokinins (zeatin), were estimated in the fresh shoot. nucleic acids (DNA and RNA), lipid peroxidation as MDA contents, H_2O_2 contents, some antioxidative enzymes as superoxide dismutase (SOD) and catalase (CAT) and protein bands were also determined in the fresh leaves. At maturity, carbohydrate contents and protein contents were analyzed in the harvested grains.

2.1. *Chemical analysis*

Total carbohydrates were determined using the method[17]. Total nitrogen was determined by using micro-kjeldahl method[18]. The protein was calculated by multiplying total nitrogen by 6.25. The method of hormone extraction was essentially similar to that adopted[19] and methylation process was carried out according[20]. Identification and determination of auxins, gibberellins, and abscisic acid were carried out by HelwettPackered gas liquid chromatography (5890) with a flame ionization detector[21].

The gas liquid chromatographic conditions for isothermal work was as follows: The chromatograph was fitted and equipped with HP–130 mmX0.32 mm X 0.25 μm capillary column coated with methyl silicon. The column oven temperature was programmed at 10_ C/min from 200_ C (5 min) to 260_C and kept finally to 10 min. Injector and detector temperatures were 260 and 300_C, 2 2 respectively. Gases flow rates were 30, 30, 300 cm/sec for N, H and air, respectively and flow rate inside 3 column was adjusted at 2 ml / min. Standards of IAA, GA and ABA were used. Cytokinin as zeatin was detected by HPLC isocratic UV analyzer ODS Hyparsil C18 column, 20 min gradient from 0.1N acetic acid. pH 2.8 to 0.1 N acetic acid in 95% aqueous ethanol, pH 4. The flow rate: 1ml/min, detection: UV 254 nm, standards of zeatin was used[22].

DNA and RNA were extracted with 10% perchloric acid following the method[23]with some modifications[24]. DNA was estimated by diphenylamine colour reaction[25], while RNA was estimated colorimetrically by the orcinol reaction[26]. The nucleic acids were determination by Spekol Spectrocolourimeter VEB Carl Zeiss The levels of lipid peroxidation were measured by determining the levels of malonadialdehyde (MDA). Malondialdhyde is a product of lipid peroxidation and was assayed by thiobarbituric acid reactive substrates (TBARS) contents[27]. H_2O_2 level was colorimetricallymeasured[28]. Antioxidant enzymes were extracted from frozen leaf tissue by using a known volume of phosphate buffer (pH 7) as described[29]. Crude extract was used for enzyme assay. Superoxide dismutase (SOD, EC 1.12.1.1) and Catalase (CAT, EC 1.11.1.6) was assayed according to the method[29]and calculated[30].

For determination of protein banding pattern, protein extraction was done according[31] with some modifications. Electrophoretic protein profile of wheat leaves were analyzed according to sodium dodocylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique[32]. Polypeptide maps, molecular protein markers, percentage of band intensity, molecular weight and mobility rate of each polypeptide were related to standard markers using gel protein analyzer version 3 (MEDIA CYBERNE TICE, USA). Total carbohydrate and protein percentages were determined in the powder of dry yield of wheat grains.

The results were statistically analyzed using MSTAT- C software[33]. The mean comparisons among treatments were determined by Duncan,s multiple range test at 5 % level of probability.

3. Results

3.1. *Growth response, total carbohydrates and protein contents of the yielded grains*

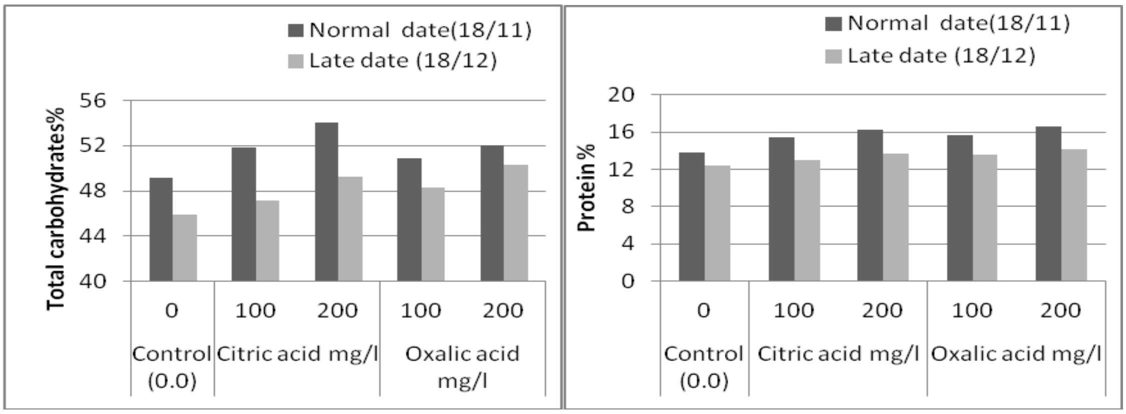
The presented data (Table 1) showed that, sowing wheat at the late date (18/12) decreased significantly growth parameters (shoot length, number of tillers/plant and dry weight of shoots per plant) as compared with that of the corresponding control sown at normal date (18/11).Late sowing of wheat reduced shoot length by 1.69%, number of tillers/plant by 16.75% and dry weight of shoot by 21.33% as compared with plants sown at suitable time.

Table 1. Effect of citric acid or oxalic acid and sowing dates on growth parameters of wheat plants at 90 days after sowing (means of two successive seasons).

Treatment		Plant height (cm)		No of tillers/plant		Shoot dry weight /plant (g)	
		Sowing date					
		Normal (18/11)	Late (18/12)	Normal (18/11)	Late (18/12)	Normal (18/11)	Late (18/12)
Control (0.0)		49.67	48.83	4.00	3.33	1.50	1.18
Citric acid mg/l	100	51.00	49.33	4.33	4.00	1.83	1.45
	200	53.33	50.17	4.67	4.33	2.35	1.75
Oxalic acid mg/l	100	54.33	51.33	5.00	4.67	2.47	1.97
	200	55.67	52.33	5.33	4.67	2.60	2.15
LSD at 5%		2.35		0.79		0.15	

Spraying wheat plants with different concentrations(100 and 200 mg/l) of citric acid oroxalic acid at thetwo dates of sowing (normal date or late date) induced significant increases in shoot length, number of tillers/plant and dry weight of shoot per plant compared to the corresponding control plants (Table 1). It is worthy to mention that, oxalic acid treatment were more effective than citric acid at the two dates of sowing, 200 mg/l oxalic acid was the most effective treatment as it gave the highest increases in all studied parameters as compared with the corresponding controls at the two dates of sowing.

Data presented in Fig (1), showed that late sowing date decreased significantly total carbohydrate% and protein% of the yielded wheat grains. The percentage of reduction were 6.75% and 10.56% for total carbohydrate% and protein%, respectively as compared with those sowed in the normal time. Concerning oxalic acid or citric acid treatments, data show that, foliar treatment with different concentrations increased significantly total carbohydrate % and protein % of the yielded grains. Citric acid with 200mg/l was the most effective treatment on total carbohydrates as it gave the highest increments of total carbohydrates%. Meanwhile, on protein contents, oxalic acid with 200 mg/l was the most effective treatmentas it gave the higest increaments of total protein % of both normal and late sowing date wheat plants.



LSD at 5%: 1.83 LSD at 5%: 1.38

Fig (1): Effect of citric acid or oxalic acid and sowing dates on carbohydrate% and Protein% of the yielded wheat grains (means of two successive seasons).

3.2. *Endogenous phytohormones*

Based on the obtained results in the first season, 200 mg/l of either citric acid or oxalic acid at normal sowing date and late date were selected for determination of endogenous hormones as shown in Table 2.It is clear from the data that IAA, GA_3 and zeatin contents showed a reduction in shoot tissues of wheat plants sowing at late date as compared with the control wheat plants sowing at suitable date in 18th november. Concerning the ABA content, it has been found in the present work (Table 2) that this content was increased in the plants sowed lately (18th December) as compared with those plants at normal date (18 November).

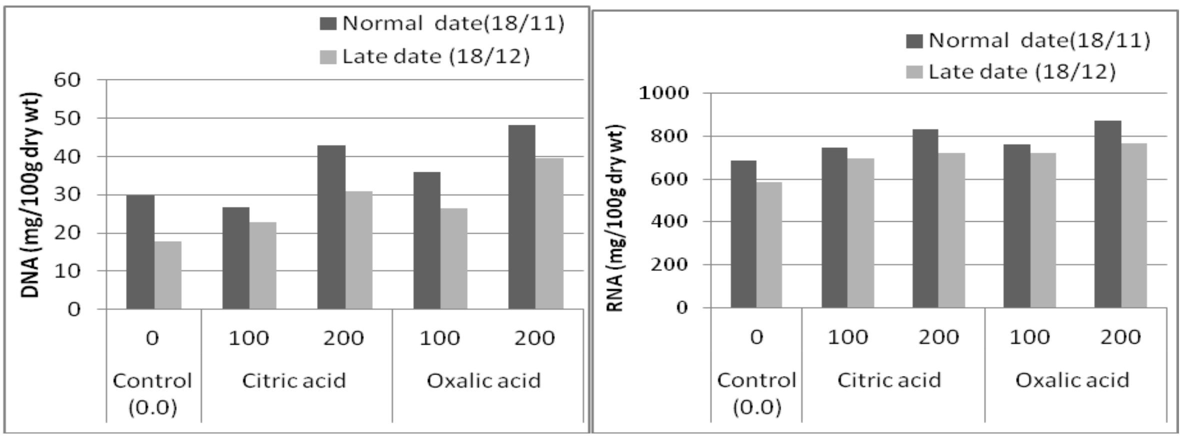
In response to citric acid or oxalic acid foliar treatments with different concentrations, data clearly show that different treatments caused marked increases in IAA, GA_3 and cytokinins. Meanwhile decreased ABA contents of wheat shoots as compared to the coressponding controls. ABA contents of the treated late sowed wheat plants still higher than control plants of normal date. Oxalic acid was more effective than citric acid (Table 2).

Table 2. Effect of citric acid or oxalic acid and sowing dates on endogenous bioregulators (IAA, GA_3, ABA and zeatin) as µg/100g fresh wt of wheat plants at 90 days after sowing.

Treatment	IAA		GA_3		ABA		Zeatin	
	Sowing date							
	Normal (18/11)	Late (18/12)	Normal (18/11)	Late (18/12)	Normal (18/11)	Late (18/12)	Normal (18/11)	Late (18/12)
Control (0.0)	32.16	9.18	197.43	114.78	22.40	91.17	69.95	11.97
Citric acid	54.23	21.64	212.57	167.70	11.31	38.49	108.18	33.24
Oxalic acid	69.31	28.05	268.24	205.32	10.26	31.22	136.39	45.81

3.3. Nucleic Acid Contents

The changes in nucleic acids (deoxyribonucleic acid DNA and ribonucleic acid RNA) in leaves extracts as a result of foliar application of citric acid or oxalic acid sowed at two different dates are presented in Fig (2). The results show significant decreases in DNA and RNA of leaves of wheat plants sowed at late time (18 December) as compared with those sowed at normal date (18 November). The percentages of decreases in DNA and RNA were 40.23% and 14.58% respectively. Foliar treatments of citric acid or oxalic acid at different concentrations induced significant increases in DNA and RNA as compared with control plant either of plants sowed at normal or late sowing date. The higher values were recorded in response to 200 mg/l oxalic acid followed by 200 mg/l citric acid.

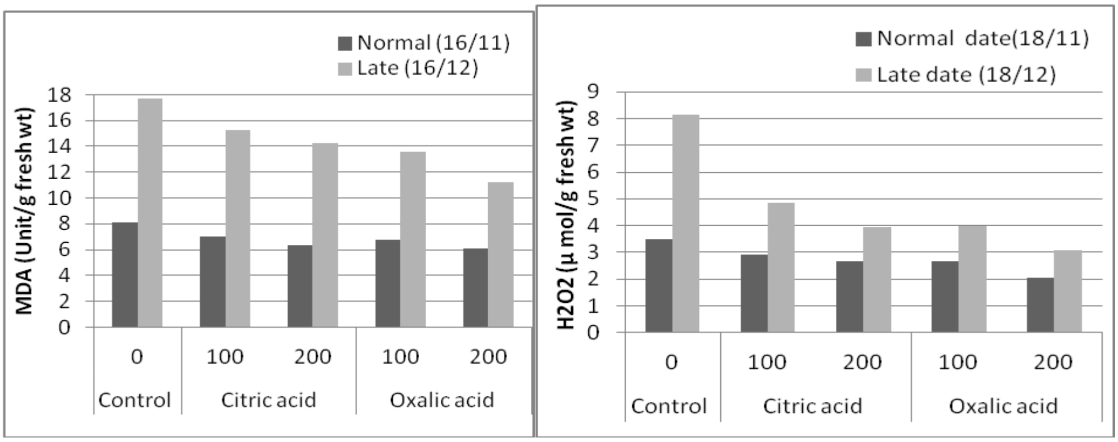


LSD at 5%:1.94 LSD at 5%:15.93

Fig 2. Effect of citric acid or oxalic acid and sowing dates on nucleic acid (DNA and RNA mg/100g dry wt) of wheat plants at 90 days after sowing(means of two successive seasons.

3.4. Lipid peroxidation and H2O2 contents

The changes in malondildhyde (MDA) and H_2O_2 contents in the leaves extracts of wheat plants treated with different concentrations of citric acid or oxalic acid and sowed in normal date or late date are presented in Fig (3). Data clearly show that sowing wheat plant at late date increased significantly malondildhyde (MDA) as well as H_2O_2 contents of wheat plants. These increments reached 118.22% in MDA content and 133.37% in H_2O_2 content in plants sowed at late date as compared with those sowed at normal time. The applied treatments of oxalic acid followed by citric acid reduced significantly MDA and H_2O_2 content compared to the corresponding control.

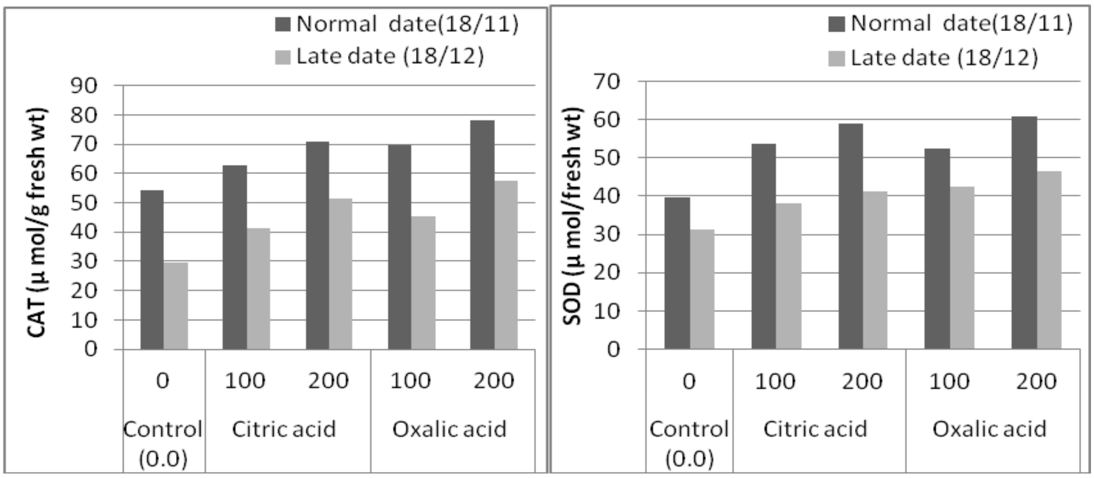


LSD at 5%: 0.989 LSD at 5%: 0.497

Fig 3. Effect of citric acid or oxalic acid and sowing dates on MDA (µ mol/g fresh wt) and H_2O_2 (µ mol/g fresh wt) of wheat plants at 90 days after sowing.

3.5. Antioxidant enzymes activities

The changes in the enzyme activities of wheat plants in response to foliar treatment with citric acid or oxalic acid at normal sowing date or late sowing date are presented in Fig (4). The reported results indicate that, CAT and SOD activities of wheat plants were reduced significantly in wheat leaves sowed at late date compared with those sowed at normal date. Late sowing caused reduction by 24.60% in CAT enzyme activity and by 20.69 % in SOD activity below the control value. Foliar spray of citric acid or oxalic acid concentrations (100 and 200 mg/l) induced significant increases in CAT and SOD activities of wheat plant either at normal sowing date or late sowing date as compared to the corresponding controls. The magnitude of increase was much more pronounced by increasing the concentration of either citric acid or oxalic acid.



LSD at 5%: 4.635 LSD at 5%: 3.396

Fig (4): Effect of citric acid or oxalic acid and sowing dates on CAT and SOD enzymes activities (µ mol/g fresh wt) of wheat plants at 90 days after sowing.

3.6. Protein electrophoretic pattern

Based on the obtained results in the first season, 200 mg/l of either citric acid or oxalic acid at normal sowing date and late date were selected for determination of protein electrophoretic pattern. The presented data (Plate 1 and Table 3) showing the changes in protein electrophoretic pattern extracted from the leaves of wheat plants in response to either citric acid or oxalic acid treatments sowed at normal and late sowing dates (18 November and 18 December). The changes in the protein electrophoretic pattern in wheat leaves sowed at late date (exposed to high temperature) exhibit an increase in the number of protein bands to 10 bands as compared to those sowed at normal date (8 bands), Table (3). Also, new proteins appeared in leaves subjected to high temperature at molecular weights 111.8, 92.21, 70.5, 45.3, 38.9, 33.1, 26.9 and 12.7 Kda and disappearance of band at 144.6, 62.6 and 36./8, 30.6 Kda. The combined effect of citric acid or oxalic acid and high temperature stress induced the appearance of 8 new protein bands their molecular weights are 111.8, 92.2, 70.5, 38..9, 33.1, 26.9 and 24.7 KDa as compared with the untreated control. This indicates that both citric acid or oxalic acid and high temperature stress share a common mechanism.

4. Discussion

Data presented in Table (1) showed that late sowing date decreased significantly growth parametersof wheat plant as compared with those sowing at normal date. Several investigators pointed to the harmful effects of late wheat cultivation and its impacts on the growth and productivity of wheat plant[34-36].These results are supported with the finding[37-39]on pea, wheat and rice plant. These reductions may be attributed to the relatively higher temperature prevailing during the critical stages of growth in late sowing plant. In addition, the reduction in the studied growth parameters as shoot length, number of tillers/plant and dry weight of shoots/plant of wheat plant in response to late sowing date can be ascribed to the effect of high temperature on the membrane permeability and the transpiration rate[40]. Application of citric acid or oxalic acid as foliar treatments caused an increase in all plant growth measurements as compared with the control treatment. Similar results were obtained by previous reviews[41-44]on different plant species. They stated that the improvement of plant growth in response to organic acids application was due to osmoregulatory role of these organic solutes which help the plants to absorb more water. Also, the improving effect of citric acid or oxalic acid on growth might be attributed to its auxinic action that was reflected on enhancing cell division as well as its effect on stimulating the biosynthesis of carbohydrates. The auxinic action of both oxalic and citric acid on enhancing cell division and cell enlargement which reflected positively on dry weight was concluded[45,46].

Late sowed wheat produced poor grains quality (decrease in both carbohydrate and protein percentage) as a result of exposing to high temperature stress during different developmental processes as compared with those sown at normal date (Fig 1). These results may be attributed to the reduction in grain weight which associated with the reduction of starch accumulation and the disruption of normal protein synthesis under high temperature stress. These results are in agreement with those obtained[47,37&38]. They reported that, protein content in grains of wheat and pea seeds significantly reduced by any duration of heat treatment. The synthesis of putative protein is largely replaced by that, of constitutive protein (heat shock protein) during a heat shock event[48]. Sudden exposure to high temperature reduced the rate and duration of protein accumulation in wheat[47].

Moreover, the reduction in the carbohydrate content in the produced wheat grains after the exposure to high temperature stress may be attributed to a reduction in endosperm cell size and a truncated duration of grain filling[49] reducing the activity of soluble starch synthase[50] or impaired initiation of α–type starch granules[51] which may be related to the reduced duration of grain filling. Concerning the effect of external supply of either citric acid or oxalic on wheat shoots sown at late date, the obtained results of citric acid or oxalic acid are in harmony with earlier reports[41,45,52,53]. They reported that percentages of total carbohydrates and nitrogen content were positively responded to spraying with oxalic acid or citric acid as antioxidant. This simulative effects of oxalic acid or citric acid might be due to that, organic acids are very important for osmotic adjustment under stress[54] and regulation of pH of plant cells[55].

Heat stress usually results in deep observed metabolic changes, which are reflected by disturbed concentration of phytohormones and further on by altered ratios between them[40].Data presented in Table (2) clearly show that late sowing date (18 Dec.) of wheat plant resulted in amarked decreases in IAA, GA_3 and cytokinins contents as compared with those plants sowing at normal date (18 Nov.). These results indicate that high temperature severly inhibits the biosynthesis of auxins, gibberellins and cytokinins and/or increase their degradation or transformation into inactive form.

The decrease in auxin, gibberellin and cytokinin contents in response to high temerature were observed[56] on *Trigonella foenum graecum* plants. Stress induced changes in rate of hormone synthesis flux and/or turnover appear to regulate the abortion process in maize kernel. The high temperature inhibitory effect on flowering is mediated through its effect on lowering level of endogenous GA and its precursors[57]. Late sowing decreased endogenous IAA, GA and cytokinins content of pea plant[37].also, late sowing of wheat plant decreased endogenous phytohormones as IAA, GA and cytokinins[58]. The increases in the endogenous growth promoting substances in response to citric acid or oxalic acid treatments might be attributed to their effect on increasing the biosynthesis of the endogenous growth promoters and / or decreasing their inactivation.

The increase in the different growth promoters could be occurred through retarding the biosynthesis of hormone degradative enzymes and / or repressing their activities or through preventing the transformation of these active substances into inactive forms. These obtained results was confirmed by those obtained[59] using antioxidant ascobin (mixture of ascorbic acid +citric acid) on wheat plant. The increases in IAA, GA and zeatin contents in shoot tissues treated with citric acid or oxalic acid paralled with the increase in growth rate (Table 1) could be attributed to the stimulation in cell division and/ or cell enlargement. Concerning the ABA content, it has been found that, this content was increased in the plant sowing in late time (Table 2). In this connection, plant growth was decreased and ABA was increased due to late sowing of pea and wheat plant[37&58], respectively. They suggested that this might be resposible for the resistance of the plant.In response to foliar treatment of citric acid or oxalic acid the reduction in ABA might be attributed to the antagonistic effect of organic acids to ABA synthesis and / or action.

Data in Fig(2) illustrates that sowing wheat plant at late time (18 December) decreased the values of DNA and RNA as compared to plants sowed at normal time. Similar results of stress have been reported[60-62]on canola, faba bean plants. The reduction of DNA and RNA contents in stressed plants may be attributed to the role of ROS which was released at high temperature stress in inducing DNase activities, enhancement of DNA fragmentation. These results are in agreement with those obtained[63] who reported that, in tobacco plants ROS detected at stress was accompanied with induction of DNase activities, enhanced DNA fragmentation and methylation. It was postulated that ROS which accumulated as a result of salt stress can damage essential membrane levels as well as nucleic acids[64].Exogenous application of citric acid or oxalic acid to wheat plants could overcome the reduction of RNA and DNA (Fig 2) i.e. foliar treatment of the used antioxidants promoted the synthesis of DNA and RNA and/or prevented their degradation by nuclease enzymes. It was reported that antioxidants as citric acid or oxalic acid reacting directly or indirectly with reactive oxygen species, so contribute to maintain the integrity of cell structure such as proteins, lipids and nucleic acids from damage which induced by heat stress[65].

Exposing wheat plants to heat temp at late sowing date induced a significant increases in MDA and H_2O_2contents over the control normal sowed plants (Fig 3). These results are in agreement with those obtained[59,61,66-70], they mentioned that exposing plant to different stress conditions as heat, cold, salinity and drought stress increased MDA and H_2O_2 contents of different plant species. High temperature stress is known to damage the cell, and alter most of its components and functions. Some of these damages caused by heat – induced oxidative stress[70].In addition the marked increases in MDA and H_2O_2 levels in different plant species may be due to inadequate induction of antioxidative and glyoxalase systems as mentioned[71].

The inhibitory effects of exogenous citric acid or oxalic acid were obtained on lipid peroxidation and H_2O_2 may be due to that antioxidant would inhibit stress-induced increases in the leakage of essential electrolytes following peroxidative damage to plasma membranes[72-74].This reduction in MDA and H_2O_2contents could also have been due to the putative role of osmolytes in alleviating the stress induced deleterious effects on the structure of cell membranes and activities of different enzymes as well as reducing the generation of highly destructive free radicals[75,76].

Antioxidant defense system in plants contains enzymatic and non–enzymatic antioxidants. The enzymatic system consists of enzymes such as catalase (CAT) and superoxide dismutase (SOD)[77]. The results of the present work showed that, the activities of the antioxidant enzymes CAT and SOD were decreased in lately sowed plants as compared with those of the normal sowed control plants (Fig 4). The activities of CAT and SOD were decreased in response to high temperature stress in bent grass and wheat plants,[38,78]. Moreover, CAT activity was decreased at supraoptimal temperature of wheat seedlings[77].It was suggested that salt stress leads to a decrease in SOD activity in salt sensitive plants and this in turn showed an increase in salt tolerance one[79]. The antioxidant enzyme system for scavenging the toxic oxygen species acts in various compartments of plant cells. Heat shock induced oxidative damage which influenced by regulating the activities of SOD in *Phaseolus vulgaris* plants[80].

Regarding to citric acid or oxalic acid treatment, it has been found in the present investigation that, exogenous application of oxalic acid followed by citric acid enhanced the activities of CAT and SOD of wheat plants as compared with those of the control plant exposed to high temperature stress without foliar treatment. These results may be attributed to the potential effect of antioxidants which act as free radical scavenger[81]. It could be concluded that, these reduction in enzyme activities could be attributed to antioxidants direct effects on scavenge ROS (O^{-2}), hydrogen peroxide (H_2O_2) and singlet oxygen ($O^{\cdot}_2$) and /or preventing the enhancement of the mentioned activated oxygen species[82].

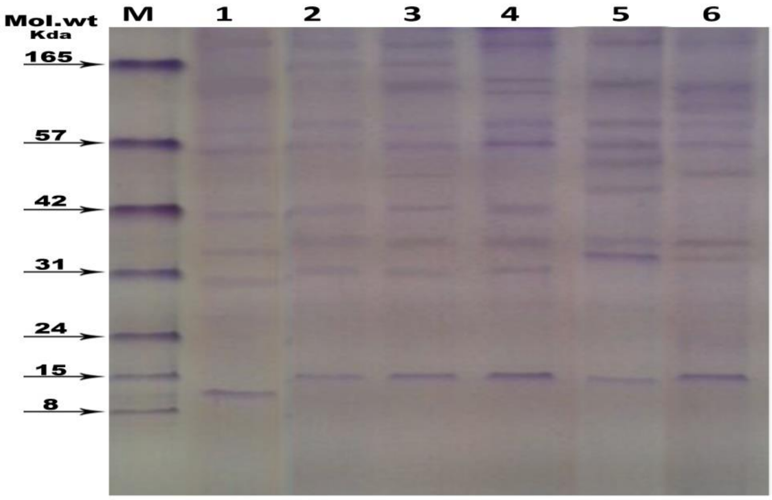


Plate 1- Electrograph of soluble protein pattern by one-dimensional SDS-PAGE showing the change of protein bands (marked by arrowheads) in response to citric acid or oxalic acid of wheat shoots sowing at normal and late sowing. Each lane contains equal amounts of protein extracted from shoots. Lane M Protein markers – Lane 1, control – Lane 2 citric acid – Lane 3 oxalic acid Lane 4, late sowing date control - Lane 5, late sowing date with citric acid - Lane 6, late sowing date with oxalic acid.

The changes in the protein electrophoretic pattern in wheat shoots sowing at late date and subjected to heat stress exhibit an increase in the number of protein bands to 10 as compared with the control (8 bands) Plate 1. The heat stress response and the heat shock proteins (HSPs) along with the correlation of HSPs expression with cellular resistance to high temperature led to the hypothesis that HSPs protect cells from the detrimental effects of high temperature and that accumulation of HSPs leads to increased thermotolerance[83].High temperature induced great significant modifications in protein pattern of wheat plant (Table 3). In this connection, it was reported that, high temperature stress induced the synthesis of polypeptides and accumulated the HSPs at different M wt. (96, 80, 70, 35 and 24 K Da.) in tobacco plants[84].Moreover,it was confirmed this effect where, new set of proteins appeared in pea shoots subjected to high temperatures at M wts 206,157, 127, 104, 91, 70, 37, 29 and 23 K Da[37].. In addition, a new set of bands appeared in shoots subjected to high temperature at molecular weights 111.2, 90.6, 70.5, 45.6, 32.4, 24.4 and 8.7 KDa confirmed these data[38] . Several evidences indicated that, the 45K Da HSPs may play a role in the development of thermo tolerance in maize plants. In addition, this HSP was synthesized over a broad temperature range starting from 35°C upwards[85]. High temperature stress induced the disappearance of protein band at M Wt. 35 K Da. in wheat plants (Table 3).

The electrophoretic pattern of proteins in wheat shoots in response to citric acid or oxalic acid treatment showed a striking difference compared with the untreated plants. The treatments induced the appearance of new ones as compared with that of the untreated plants (Table 3). The promotive effect of organic acids in ameliorating the high temperature injury via inducing the synthesis of heat shock proteins may be attributed to the role of organic acids in improving the stability of enzyme or protein molecules which were affected by heat stress.

Table 3: Effect of foliar treatment of citric acid or oxalic acid on protein bands of wheat plant at normal and late sowing date at 90 days after sowing.

M wtKda	Normal date			Late date		
	Control	Citric acid	Oxalic acid	Control	Citric acid	Oxalic acid
206.5	+	+	+	+	+	+
165.2		+	+			
144.6	+	+	+			
111.8				+	+	+
92.2				+	+	+
70.5				+	+	+
62.6	+	+	+			+
57.0	+	+	+	+		
45.3	+	+	+	+	+	
38.9				+	+	+
36.8	+	+	+		+	+
33.1		+	+	+		+
30.6	+	+	+		+	
26.9				+	+	+
24.65					+	+
12.68	+	+	+	+	+	+
Total bands number	**8**	**10**	**10**	**10**	**11**	**11**

Conclusion

It could be concluded that foliar application of citric acid or oxalic acid could play anenhancement role and alleviate the harmful effect of late sowing stress on many metabolic and physiological processes of wheat plant that reflected in increasing seed yield quality. Oxalic acid has gained the most tolerance capacity mainly through inducing the highest endogenous hormones, DNA, RNA contents, antioxidant enzymes (SOD and CAT) activities, number of new responsive protein bands, the lowest MDA and H_2O_2 contents. Whereas the quality of the yielded wheat grains has being better responded by citric acid rather than oxalic acid.

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