ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.09 pp 124-131, 2016

Physiological responses of grain soaking with Aspirin on two cultivars of wheat plant

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Abstract : A pot experiment was carried out at the green house of National Research Centre, Dokki, Egypt, during two winter seasons of 2013/ 2014 and 2014/ 2015. To investigate the physiological role of Aspirin (acetyl salicylic acid) with different concentrations on growth, some biochemical aspects and yield of two wheat cultivars (Giza 168 and Giza 10). Wheat grains were soaked for 6 hours in different concentrations of Aspirin (2 mM and 4 mM). The obtained data show that, Aspirin treatment improved wheat plant growth and yield via increasing growth parameters (plant height, number of tillers and leaves /plant and dry weight of plant), antioxidant enzymes (polyphenol oxidase PPO, phenyl alanineammonia lyase PAL, catalase CAT and peroxidase POX), total phenol contents, antioxidant activities (DPPH) and nucleic acids contents (deoxyribo nucleic acid DNA and ribonucleic acid RNA). Data clearly show that, higher concentration (4 mM) of Aspirin was more effective than lower concentration (2 mM) in improving plant growth parameters, some biochemical aspects and yield parameters (spikes number/plant, spikes weight/plant and grains weight/plant).
Key words : Aspirin, biochemical aspects, DPPH activity, growth, Wheat, yield.

Introduction

Wheat (*Triticum aestivum* L.) is a very important cereal crop all over the world, after maize and rice wheat is considered the third cereal crop cultivated, meanwhile, it considered the second cereal after rice as the main food crop regarding to dietary intake. Wheat crop is a hardy crop so it can grow in different environmental conditions thus lead to large scale cultivation as well as storage of crop for long time. About 70% of this crop is used for food, about 19% as animal feed and the 11% remaining for used in industrial applications as biofuels. Grains of wheat considered the basic ingredients of bread and other bakery products in addition to pastes as wheat grains can be ground into flour semolina, etc. So, it considered the main source of nutrients for most of world populations Abd Allah et al[1,] Yu et al [2]. Also, wheat grains helps in prevention of diseases and health promotion as it is an excellent source of natural antioxidants and its high nutritional values Abd El-Rheem et al[3,] Hussein et al [4]. However of this importance of wheat grains its local production is not sufficient for the annual demands. Thus increasing local production is the main target to overcome this deficiency. This might be reached via using varieties with more productivity, enhancing practices of culture as well sowing in newly reclaimed soils or using some growth regulators as seed presoaking or foliar treatments during different growth stages Singh and Pal [5]. In Egypt, wheat sown at (1-15) November the normal sowing date, and it might be exposed to high temperature stress during the stage of grain filling (at March or April) due to El-Khamaseen hot wind for one or more days thus in turn reduces growth, yield and quality of grains mainly by shortening the reproductive and ripening growth phases Sadak and Orabi [6,] Hamouda et al [7].

Presoaking is one of different strategies to increase plant production, seed priming (pre-sowing seed treatment) is low cost, low risk technique and easy technique that help in raising plant productivity under different environmental conditions Sadak [8,] Bakhoum & Sadak [9]. Phytohormones as Salicylic acid is one of plant growth regulators used in enhancement of plant production. Pre sowing treatment with salicylic acid has been shown to be useful for germination, growth and yield of different crops Kaur et al [10]. The enhancement role of seed presoaking with different growth bioregulators on plant growth and grain yield might be due to the their involvement in increased rate of photosynthate translocation from leaves to grains Kiseleva and Kaminskaya [11]. Priming of seeds enhance germination speed and uniformity as well as stimulates different biochemical changes in seeds that are vital in breaking dormancy, enzyme activation, hydrolysis and mobilization of seed reserves and the emergence of embryonic tissues Catav et al[12].

Salicylic acid (SA) is a widespread phenolic compound and an endogenous growth regulator that regulate many physiological processes in plant and known as a potential signal molecule for modulating responses of plants to different environmental stresses Sakhabutdinova et al [13]. SA have a synergetic effect on plant growth, induction of flowers, control the uptake of ions by roots and stomatal movement in plants, affects on biosynthesis of ethylene and reverse abscisic acid effects on leaf abscission. Moreover, enhancing chlorophyll and carotenoids pigments biosynthesis, rate of photosynthesis and modifying the activity of some important enzymes levels Sadak et al [14]. Also, SA is an important non enzymatic antioxidant that plays an important role in some physiological processes regulation in plant species. SA affects on different biochemical processes of plants depends on SA concentrations, plant growth stage at which SA added, environmental conditions and type of plant. Acetyl salicylic acid and salicylic acid application to pea plants improved plant growth as plant height, leaves number, fresh & dry weights El-Shraiy and Hegazi [15], Kamel et al [16]. Foliar treatment of salicylic acid improved growth and yield of different plant species Abd El-Hamid and Sadak [17,] Dawood et al [18,] Orabi et al [19,] Orabi et al [20,] Sadak and Abd Elhamid [21] on different plant species.

So, this study was undertaken to study the physiological role of acetyl salicylic acid (asprin) on growth, some biochemical aspects and yield of two cultivars of wheat plants.

Materials and Methods

A pot experiment was carried out at two successive winter seasons 2013/2014 and 2014/2015 in the green house on National Research Centre, Dokki, Giza, Egypt to study the effect of soaking of wheat grains in different concentrations of Aspirin on growth, some biochemical aspects and yield attributes of wheat plants. Wheat (*Triticum aestivum* cv. Giza 168 and Giza 10) grains were obtained from Agricultural Research Centre, Ministry of agriculture and Land Reclamation, Arab Republic of Egypt. Wheat grains were soaked in different concentrations of Aspirin (0. 2 and 4 mM) for 6 hours. The experiment were carried at 15^{th} and 16^{th} of November in the two successive seasons, 10 uniform air dried grains of wheat were sown along a centre row in 50 cm diameter earthen – ware pots containing equal amounts of clay soil. Wheat grains were sown in complete randomized design. Fertilization were carried out with the recommended doses 5g phosphorous/pot as triple phosphate, 6 g nitrogen/pot as urea and 5 g potassium/pot as potassium sulphate during preparation of pots and after sowing. Watering was carried out according to the usual practice. Thinning was done after 15 days from sowing as five uniform seedlings were left in each pot. Plant samples were taken after 90 days from sowing for measurement of growth parameters and some biochemical aspects. Some plant leaves were stored in deep freezer at -20°C until used for estimation of some antioxidant enzymes (poly phenol oxidase (PPO), phenyl alanine ammonia lyase (PAL), catalase (CAT) and peroxidase (POX), phenolic contents and DPPH activity. The rest of plants were weighted and dried in an electric oven at 70°C till constant weight for estimation of dry weight, and air dried for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). At harvest, measurements of the yield and its components were recorded (160 days after sowing) as (spikes numer/plant, spikes weight/plant and grains weight/plant).

Chemical analysis:

Analysis of enzyme activities:

The method used for extracting the enzymes was described by the method of MuKherjee and Choudhuri [22]. Polyphenol oxidase (PPO; EC 1.10.3.1) activity was assayed using the method of Kar and Mishra[23]. The activity phenyl alanine ammonia lyase (PAL, EC, 4.3.1.1) enzyme was carried out according

Beaudoin et al [24]**.** Catalase (CAT; EC 1.11.1.6) activity assayed according to the method of Chen et al [25]. Peroxidase (POX; EC 1.11.1.7) activity assayed using the method adopted by Bergmeyer[26]. The enzyme activities were measured by using the Spekol Spectrocolorimeter (Carl Zeiss AG; Jena, Germany).

Estimation of total phenols:

Total phenols contents was extracted and determined as described by Daniel &George [22].

Determination of DPPH activity:

The free radical scavenging activity was determined (Liyana-Pathiranan and Shahidi [28]) using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent.

Estimation of Nucleic acid contents:

DNA and RNA were extracted with 10% perchloric acid following the method adapted by Schmidt & Thannhauser[29] with some modifications as described by Morse & Carter[30]. DNA was estimated by diphenylamine colour reaction (Burton[31]), while RNA was estimated colorimetrically by the orcinol reaction (Dische[32]). The nucleic acids were determination by Spekol Spectrocolourimeter VEB Carl Zeiss

Statistical Analysis:

The results were statistically analyzed using MSTAT-C software (MSTAT-C[33]). The mean comparisons among treatments were determined by Duncan's multiple range test (Gomez & Gomez[34]) at 5 $P \leq 0.05$.

Results

Plant growth parameters : Table (1) presented the effect of soaking wheat grains in Aspirin different concentrations (0.0, 2 mM or 4 mM) on growth parameters as plant height (cm), number of tillers & leaves / plant and dry weight of shoot/ plant (g). Data clearly show that, treatment wheat plant with Aspirin increased significantly most of the above mentioned growth parameters of the two cultivars (Giza 168 and Giza 10) as compared with control plants. These increases were gradually with increasing Aspirin concentrations thus 4 mM concentration of Aspirin was the most effective treatment in both tested cultivars. Giza 10 cultivar was superior to Giza 168 cultivar in all the studied morphological parameters.

Table (1): Effect of Aspirin on morphological parameters of two cultivars of wheat plant (Giza 168 and Giza 10). Data are means of two seasons.

Cultivar	Giza 168			Giza 10		
Aspirin (mM)	0.0	2 mM	4 mM	0.0	2 mM	4 mM
Plant height (cm)	61.00^{d}	63.33^{c}	64.50^{c}	73.0^{b}	76.67^{a}	78.33^{a}
Tillers number/plant	2.67^{d}	3.67^{c}	4.33^{b}	3.67^{c}	4.67^{ab}	5.33^{a}
Leaves number/plant	10.67^{d}	11.33^{cd}	13.00^{c}	12.33^{cd}	13.67^{b}	14.67^{a}
Dry wt of shoot/plant (g)	1.85^{d}	1.94^{cd}	2.11^{cd}	2.15^{c}	2.62^{b}	3.22^{a}

Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Activities of antioxidant enzymes:

Plants have different antioxidant system such as enzymatic antioxidants as peroxidase (POX), catalase (CAT), phenyl alanine ammonia lyase (PAL) and poly phenol oxidase (PPO). Table (2) show that, soaking of wheat grains with Aspirin different concentrations (2 & 4 mM) induced significant increases in different enzyme activities as POX, CAT, PAL and PPO as compared with control untreated plants. These increases were gradual increases with increasing Aspirin concentrations from 2mM to 4 mM.

Table (2): Effect of Aspirin on antioxidant enzymes (Unit/min/g/f wt) of two cultivars of wheat plant (Giza 168 and Giza 10) Data are means of two seasons

Cultivar	**Giza 168**			**Giza 10**		
Aspirin (mM)	0.0	2 mM	4 mM	0.0	2 mM	4 mM
POX	13.97[d]	17.26[c]	18.64[b]	16.32[c]	19.21[b]	23.45[a]
CAT	55.11[e]	58.61[d]	68.80[b]	63.77[c]	66.89[b]	81.23[a]
PAL	17.61[d]	18.48[cd]	19.74[c]	21.84[b]	23.75[ab]	24.80[a]
PPO	14.93[e]	16.54[de]	18.41[cd]	18.90[bc]	21.16[b]	27.71[a]

Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Changes on phenolic contents:

Data presented in Table (3) show the effect of grain soaking of wheat two cultivars on phenolic contents of plant. Data clearly show that different concentrations of Aspirin (2 mM or 4 mM) increased significantly phenolic contents of the two cultivars as compared with the corresponding controls. Higher concentration (4 mM) of Aspirin was more pronounced than lower concentration (2 mM) as it caused 29.80% and 46.07% compared with 19.21% and 38.76% of Giza 168 and Giza 10 respectively.

DPPH antioxidant activity:

Table (3) presented the results of free radicals scavenging activity (DPPH) of wheat plant extracts of the two cultivars (Giza 168 and Giza 10). The Table show that Aspirin treatment to wheat plant increased significantly DPPH antioxidant activity of the two used cultivars. Soaking wheat grains with 4 mM Aspirin was more effective than 2 mM as higher concentration caused 46.68% of increases and 39.22% of increase in comparison with 22.20% and 24.58% of lower concentrations of cultivar Giza 168 and Giza 10 respectively as compared with untreated controls.

Changes in DNA and RNA contents:

Data presented in Table (4) added also, that increasing Aspirin concentration from 2 mM to 4 mM significantly increased RNA & DNA contents in the two wheat cultivars (Giza 168 & Giza 10). The percentages of increases in RNA contents changed from 24.22 % to 39.68% in Giza 168 cultivar and from 14.29% to 38.10% in Giza 10 cultivar. Moreover the percentage of increases in DNA contents increased from 24.22% to 39.84% in cultivar Giza 168 and from 13.38% to 38.03% of cultivar Giza 10 as compared with their corresponding controls.

Table (3): Effect of Aspirin on total phenols (mg/g fresh wt), DPPH%, RNA and DNA (mg/g dry wt) of two cultivars (Giza 168 and Giza 10) of wheat plant (Data are means of two seasons)

Cultivar	**Giza 168**			**Giza 10**		
Aspirin (mM)	0.0	2 mM	4 mM	0.0	2 mM	4 mM
Total phenols (mg/g fresh wt)	1.51[d]	1.80[b]	1.96[b]	1.78[c]	2.47[a]	2.60[a]
DPPH %	25.13[d]	30.71[c]	36.86[b]	29.37[c]	36.59[b]	40.89[a]
RNA (mg/g d wt)	1.28[d]	1.59[c]	1.79[b]	1.47[c]	1.68[b]	2.03[a]
DNA (mg/g d wt)	1.23[d]	1.53[c]	1.72[b]	1.42[cd]	1.61[bc]	1.96[a]

Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Yield and yield attributes:

As presented in Table (4) different concentrations of Aspirin had stimulative effect on yield and its attributes (spikes number/plant, spikes weight / plant and grains weight / plant). These increases were gradually by increasing Aspirin concentration from 2 to 4 mM. The percentages of increases increased from 16.75% to 25.0%, 15.42% to 21.66% and 39 for cultivar Giza 168 and increased from 6.38% to 25.14%, 4.39% to 11.58%

and 8.89% to 23.87% in cultivar Giza 10 for spikes number/plant, spikes weight / plant and grains weight / plant.

Table (4): Effect of Aspirin on yield and yield attributes of two cultivars (Giza 168 and Giza 10) of wheat plant (Data are means of two seasons)

Cultivar	Giza 168			Giza 10		
Aspirin (mM)	0.0	2 mM	4 mM	0.0	2 mM	4 mM
Spikes no/plant	4.0[d]	4.67[c]	5.0[bc]	5.33[b]	5.67[b]	6.67[a]
Spikes wt/plant (g)	5.77[e]	6.66[d]	7.02[c]	7.51[b]	7.84[b]	8.38[a]
Grains wt/plant (g)	3.61[e]	5.02[d]	5.27[cd]	5.74[c]	6.25[b]	7.11[a]

Means with different letters above bars were significantly different at the 0.05 level according to Duncan's multiple range test.

Discussion

One of most effective techniques for improving crop productivity is by using exogenous application of some potential growth regulators. Salicylic acid is one of these growth regulators and induce significant effects on different physiological aspects in plants, one of salicylic acid compounds is Aspirin As these compounds affect on plant in variable manner, enhancing certain processes and inhibiting others (Raskin [35]). The obtained results showed the stimulatory effects on vegetative growth parameters of wheat plant cultivars (Table 1). It was found that, soaking sunflower seeds in salicylic acid improved growth parameters of sunflower cultivars (Dawood et al [18]), Others confirmed the obtained results[36&37] on different plants, respectively, also, it was noticed that, acetyl salicylic acid pretreatment increased growth parameters of barley plant (Kabiri and Naghizadeh [38]). This stimulative effect of Aspirin on wheat two cultivars might be resulted from its stimulatory effect on photosynthesizing cells (Zhoi et al [39]). As well as the increases in dry weight of shoots of the two cultivars of wheat plant could be attributed to the increased number of tillers and leaves per plant that lead to increased photosynthetic processes. In addition this promotive role of Aspirin might be resulted from its bioregulator roles on various biochemical & physiological processes in plant such as regulation of sink / source, cell elongation, division, and differentiation, biosynthesis of proteins, activities of different enzymes, and increase in antioxidant capacity of plants (Blokhina et al [40]).

The antioxidant enzyme systems (such as POX, CAT, PAL and PPO) of wheat plant increased in response to grain soaking with Aspirin different concentrations of the two cultivars as compared with control plants (Table 2). These increases might be attributed to the Aspirin direct function in enhancing active oxygen species (AOS) such as H_2O_2 So the increases in antioxidant enzymes activities increases for decomposing the harmful AOS (Wendehenne et al [41]). Similar results were obtained by Orabi et al[42] on cucumber plant, Orabi et al[19&20] on faba bean & tomato and Gholamnezhad et al[43] on wheat plant. With regard to PAL enzyme contents of wheat leaves in this study, the close relationship between the higher activities of PAL and the higher levels of phenol contents of wheat plants was conducted (Table 2 & 3). In controlling biosynthesis of phenols from phenylalanine, PAL is one of the key enzymes. PAL enzyme catalyzes the elimination of ammonia from phenylalanine to give trans-cinnamic acid. This reaction is the first step of phenyl propanoid pathway in plants which results in the diversion of L-phenylalanine into secondary metabolism with subsequent production of phenolic compounds (Hassanein et al[44]).

Regarding to phenolic contents in the present study, soaking grains of the two wheat cultivars with Aspirin increased phenolic contents of the two cultivars (Giza 168 and Giza 10) as compared with control plants. Moreover, these increases in phenolic contents could be due to that phenols act as a substrate for many different antioxidant enzymes (Lewis and Yamamoto[45]). In addition to the other role of this antioxidative phenolics properties is the phenolic ability to decrease fluidity of membrane (Bakry et al [46]). Data of increased phenolic contents in response to Aspirin treatment are in agreements with the earlier results using t-cinnamic acid on roselle plant by Sadak et al [47], using trans cinnamic and benzoic acid on termis plant by El-Awadi et al[48]. These increases in phenolic contents might be one aspect of salicylic acid or Aspirin role as plant growth regulators Pokorny [49]. Moreover, these increases could be due to phenolic contents role as an efficient mechanism in different plant metabolic processes regulation and resulted consequently overall plant growth[35] With respect to yield and yield attributes of the two cultivars of wheat plant in response to different concentrations of salicylic acid treatment, Table (4) show that soaking wheat grains of the two cultivars (Giza 168 and Giza 10) in Aspirin different concentrations generally, increased significantly yield and yield attributes. These results are confirmed with the earlier obtained data of many investigators (Merwad et al [50], Rady et al [51] & Hussein et al [52]) on different plant species. These increases in yield might be a reflection of the increased growth and development and also, these increases could be due to Aspirin (salicylic acid) effect on improving growth that followed by stimulation of some physiological processes and translocation of the product of photosynthesis from source to sink[21].

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