

Improvement of Productivity and Quality of two Wheat Cultivars by Foliar Application of Spermine and Paclobutrazol

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ABSTRACT

Two pot experiments were conducted during two growing seasons 2011/2012 and 2012/2013 at the green house of the National Research centre, Dokki, Giza, Egypt to study the effect of some growth regulators (spermine and paclobutrazol) on growth, yield and some biochemical constituents of two wheat cultivars (Giza 168 and Gimeza 9). Spermine and paclobutrazol concentrations (20 and 40 mgL⁻¹) were applied during the vegetative stage. Both growth regulators specially 40 mgL⁻¹ for spermine and 20 mgL⁻¹ for paclobutrazol significantly improved plant growth parameters (number of tillers/plant, number of leaves/plant and dry weight of shoots/plant) during the vegetative stage. On contrary, 40 mgL⁻¹ followed by 20mgL⁻¹ paclobutrazol scored the greatest reduction in plant height and thus might improve lodging resistance. The greatest values for yield components (spikes No/plant, spikes wt/plant, grains wt/plant, grains No/plant, grains No/spike and 1000 grain wt) were gained by 40 mgL⁻¹ followed by 20 mgL⁻¹ spermine, then 20 mgL⁻¹ paclobulrazol. Spermine was more effective than paclobutrazol in improving the nutritional values of yielded grains and the all studied traits were being better responded by Gimeza 9 than by Giza 168.

Key words: Wheat, growth regulators, Spermine, Paclobutrazol, growth, yield, biochemical.

Introduction

Wheat (*Triticum aestivum*, L.) is one of the main cereal crops in the world as well as in Egypt. Improvement of wheat yield is the primary concern of plant physiologists, plant breeders and soil experts. The grains of wheat contain large amounts of carbohydrates, proteins, in addition to some minerals and vitamins. For the economical value of wheat, efforts aiming for further increase in wheat production are still going on, and devoted for wheat production. Increasing plant productivity either in the form of dry weight or yield, is one of the main targets in Egypt's agricultural policy, this could be achieved through fertilization and/ or growth regulators treatment including promotors and retardants.

In Egypt, wheat sown at normal sowing date (1-15) November, may be exposed to high temperature stress during grain filling (at March or April) due to the hot wind of El-Khamaseen for one or more days which in turn reduces growth, yield and quality of grains mainly by shortening the reproductive and ripening growth phases (Nagarajan and Rana, 2002, Singh and Pal, (2003).

Recently, a great attention has been focused on the possibility of using natural and safety substances in order to overcome this problem and improve plant growth and production, plant growth regulators are one of these compounds. Polyamines (PAs: putrescine, spermidine and spermine) are small aliphatic polycations that are classified as growth regulators (Smith, 1985, Evans and Malmberg, 1989, Ayad *et al.*, 2010). Polyamines are part of the overall metabolism of nitrogenous compounds and influence the transcriptional and translational stages of protein synthesis (pegg, 1986), stabilize membranes (Schuber, 1989), regulate gene expression for key enzymes of their biosynthesis and degradation (Kuznetsov *et al.*, 2006). A variety of roles have been also proposed for PAs, including cell division, root growth, fruit development, apoptosis, morphogenesis, floral initiation and development (Paschalidis and Roubelakis – Angelakis, 2005) , Liu *et al* 2006, Pang *et al.*, 2007, khan *et al.*, 2012). In addition to their role in plant development, PAs may also play an important role in plant stress responses (Al Cazar *et al.*, 2010). It is widely reported that PAs concentration inside the cell is very responsive to external conditions, and a rapid change in light, temperature and various environmental stress agents may increase PAs levels many folds (Galston and Kaur- Sawhney, 1995, Liu *et al.*, 2000).

The term growth retardants is used for all chemicals that retard cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects (PGRSA, 2007). One of the most widely used growth retardants is paclobutrazol (pp333)-1- (4-chlorophenyl-4,4 – dimethyl-2- (1H-1,2,4 triazol-1-y1) pentan-3-01] is a well-known plant growth retardant (Davis and Andersen, 1998). Paclobutrazol is a member of the triazole family of plant growth regulators, has been found to protect several crops from various environmental stresses, including drought, chilling, heat and UV. radiaiton (280-320nm). (Lurie *et al.*, 1994,

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Pinhero and Fletcher, 1994, Kraus *et al.*, 1995 and Orabi *et al.*, 2010). Paclobutrazol functions by inhibiting cytochrome P-450, which mediates oxidative dimethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellins biosynthetic pathway (Fletcher *et al.*, 2000). The characteristic effect of paclobutrazol is the inhibition of vegetative growth (manifested by reduction in the height of plant and its individual internodes) resulting in compact and sturdy plants, so, growth retardants were employed to reduce crop lodging (Jung and Rademacher, 1983) arises at the harvest stage from the exposure of plants to storms and tempests limiting the yield of wheat grains especially that grown in newly reclaimed lands and in soils where water logged during growing seasons (Orabi, 1994).

The aim of this work was to study the role of spermine or paclobutrazol on growth, biochemical changes and yield of wheat plants.

Materials and Methods

A pot experiment was conducted in the green house of National Research Centre, Dokki, Giza, Egypt during two successive growth seasons 2011/2012 and 2012/2013 to study the effect of foliar application of different concentrations of spermine or paclobutrazol on growth, yield and some physiological and biochemical constituents of wheat plants.

Wheat (*Triticum aestivum* cv. Gimeza 9 and Giza 168) grains obtained from Agricultural Research Centre, Ministry of Agriculture, Arab Republic of Egypt were sown on 15th and 16th of November in two successive seasons in 50cm diameter earthen — ware pots (at a density of 10 grains /pot) containing equal amounts of clay soil . Fertilization was done with the recommended doses i.e (5g phosphorous/pot as triple phosphate, 6g 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. After 15 days from sowing thinning was carried out, so as five uniform seedlings were left in each pot.

The pots were divided into two sets for the two cv. Each set was divided into 5 groups, each group composed of 10 pots. The plants of the 5 group were sprayed with H₂O (as control), 20, 40 mgL⁻¹ spermine and 20, 40 mgL⁻¹ paclobutrazol respectively in the two cv. These treatments were carried out twice at 30 and 37 days after sowing. The plant samples were taken after 75 days from sowing for measurements of growth parameters. Some plant leaves were stored in deep freezer at -20°C until used for estimation of total phenol and PAL enzyme activity. The rest of the plants were weighted and dried in an electric oven at 70°C till constant weight, for estimation of dry weights. Measurements for the yield and its components were also recorded at the harvest (160 days from sowing) and estimations of some biochemical constituents of yield grains were occurred.

Biochemical analysis: Total phenols were determined colorimetrically by using Folin Ciocaltea reagent according to Singleton *et al.*, (1999). The activity of phenylalanine ammoniolyase (PAL, EC 4.3.1.5), extracted and assayed according to the method adopted by Beaudoin – Egan and Thorpe (1985). Carbohydrate constituents were extracted by overnight submersion of dry tissue of the yielded grains in 80% (v/v) ethanol at 25°C with periodic shaking. Glucose content was estimated using O-toluidine procedure of Feteris (1965). Sucrose was determined using the modification of Handel (1968). Total soluble sugars were analyzed according to the modification of Yemm and Willis (1954). The method used for estimation of polysaccharides was that of Thayermanavan and Sadasivam (1984). Determination of total carbohydrates was carried out according to Herbert *et al.*, (1971). The method used for the extraction of nitrogenous constituents was essentially that adopted by Yemm and Willis (1956). The method used for determination of amino – N was designed by Muting and Kaiser (1963). Total soluble nitrogen was determined by the conventional semi micro-modification of Kjeldahl method of Pirie (1955). Total nitrogen was determined by the conventional semi micro modification of Kjeldahl method of Chinbal *et al.* (1943). Protein-N was determined by Subtracting total soluble-N from total-N. Protein content was determined by mickkjeldahl method according to Miller and Houghton (1945).

Statistical Analysis:

The results were statistically analyzed using MSTAT-C (1988) software. The mean comparisons among treatments were determined by Duncan's multiple range test at $5 P \leq 0.05$. (Gomez and Gomez, 1984).

Results and Discussions

Effect on vegetative growth:

Data recorded on vegetative growth traits i.e plant height, number of tillers/ plant, number of leaves/ plant and dry weight of shoots/ plant as affected by spermine and paclobutrazol (PP333) at 20 & 40 mgL⁻¹ are presented in Table (1). Foliar application of various concentrations of spermine or paclobutrazol caused gradual reduction in plant height of plants of the two studied wheat cultivars; the magnitude of reduction is parallel to the concentration as compared to the corresponding controls. The highest concentration of PP333 (40 mgL⁻¹) was the most effective one for producing the shortest plants in comparison with the corresponding controls.

Such retardant effect was due to reduction of internode length (Lecain *et al.*, 1986). Parallel to these results of spermine treatments, Abd El- Monem (2007) found that foliar application of various concentrations of putrescine at 30 DAS induced a marked significant reduction in shoot length when the samples were harvested at 75 days old plants as compared with similar treatments sprayed at 60 DAS.

The retardation of plant height may be attributed to inhibition of ent-kaurene oxidase, which catalyzes the sequential oxidations from ent-kaurene to kaurenoic acid in the early sequence of GA biosynthesis (Yamaji *et al.*, 1991). When gibberellins production is inhibited, cell division still occurs, but the new cells do not elongate, (Fletcher *et al.*, 2000, Taiz and Zeiger, 2006).

Paclobutrazol can also be effective for obtaining sturdy plant and reducing plant height and lodging in several species without decreasing flowering quality and improves resistance to environmental stress conditions (Rademachen, 2000; Berova *et al.*, 2002; Mansuroglu *et al.*, 2009; Currey and Lopez, 2010).

On contrary, tillers and leaves number/ plant and dry weight of shoot / plant were almostly increased by spermine treatments and the lowest PP333 treatment (20 mgL⁻¹) as compared to the corresponding controls in the two wheat cultivars. Moreover, the heaviest dry weights of shoots / plant were gained by 40 mgL⁻¹ spermine sprayed plants. The response was more obvious at Gimeza 9 than at Giza 168.

In this concern, polyamines could be act as activators of physiological processes in plant (Imai *et al.*, 2004). Paschalidis and Roubelakis – Angelakis, (2005) reported that polyamines, their precursors and their biosynthetic enzymes were correlated with cell division, expansion, differentiation and vascular development in tobacco plant.

Moreover, Abd El Wahed and Gamal El Din (2004) mentioned that spermidine as one of the polyamines, stimulated vegetative growth characters of chamomile plants, Also, putrescine enhanced the growth (fresh and dry weight) of *mentha piperita* (Youssef *et al.*, 2002), wheat plant (El-Bassiouny *et al.*, 2008) and sunflower plant (Sadak *et al.*, (2012). Putrescine significantly promoted vegetative growth characters in addition to significant increases in dry matter contents of geranium occurred in the two cuttings (Ayad *et al.*, 2010) and Jojoba plants (Taha *et al.*, 2015)

Regarding to paclobutrazol, the remarkable increments in tillers number might be attributed to the high levels of cytokinins accompanied by low level of IAA which led to weakness of apical dominance whereas, dry matter accumulation was clearly decreased by increasing triazole concentration (Bekheta *et al.*, 2003; Orabi *et al.*, 2010) where it may prevent excessive vegetative growth and improve translocation of photosynthates from source to sink (Al-Abdul Kreem , 1993, Mekki and Orabi, 2007).

Our results showed similar trend to those obtained by many investigators working on triazoles (Orabi *et al.*, 2010 on cucumber, Ribeiro *et al.*, 2011 on sun flower; JungKlang and Saengnil, 2012 on Patumma).

Table 1: Effect of foliar treatment of spermine and paclobutrazol on vegetative growth traits of two cultivars of wheat (Data are means of two seasons).

Cultivar		Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9
Treatments		Plant height (cm)		No of tillers/plant		No of leaves/plant		Dry wt. of shoots/plant (g)	
Control		59.17 ^a	57.83 ^a	2.67 ^d	2.78 ^d	12.78 ^b	14.22 ^{ab}	1.75 ^{bcd}	1.86 ^{abcd}
Spermine	20	56.00 ^{ab}	56.83 ^a	3.33 ^c	3.78 ^{bc}	13.00 ^b	14.78 ^a	1.85 ^{abcd}	1.96 ^{ab}
	40	54.17 ^{abc}	56.00 ^a	3.78 ^{bc}	4.22 ^b	13.78 ^{ab}	14.89 ^a	1.93 ^{abc}	2.04 ^a
Paclobutrazol	20	49.00 ^d	51.83 ^{bcd}	4.22 ^b	5.11 ^a	10.45 ^c	15.22 ^a	1.70 ^d	1.95 ^{ab}
	40	44.83 ^{de}	49.17 ^d	4.11 ^b	5.03 ^a	9.78 ^c	10.22 ^c	1.47 ^e	1.73 ^{cd}

Effect on yield components:

Yield as represented by spikes number/plant, spikes weight/ plant, grains weight/ plant, grains number/plant, grains number/ spike and 1000 grain weight were increased significantly by 40 mgL⁻¹ spermine (Table2). Obvious increments at 20 mgL⁻¹ paclobutrazol, whereas decrements were attained at the highest concentration of paclobutrazol (40 mgL⁻¹).

These increments in yield components due to spermine treatments may be attributed to the increase in growth rate. In this respect, Davis (1995) reported that polyamines play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits, due to the fact that polyamines, and spermine with special concern, have the ability to increase the efficiency of solar energy conversion into different photosynthetic outputs which maximized the growth rate of wheat plant and consequently increased its productivity and yield components. Additionally, spermine may exert a stimulatory effect on wheat plants through their role in increasing the endogenous phytohormones (In particular cytokinins) which in turn increase the yield components through breaking the apical dominance of wheat plant leading to the increase in flowering tillers and consequently the number of spikes and their weight and/ or through increasing the assimilates and their translocations from leaves to spikes as the spike weight

increased (El-Bassiouny *et al.*, 2008) and developing grains particularly after looking to its effect on phloem area in both flag leaf and its peduncle (Aldesuquy *et al.*, 2013).

Our obtained data of polyamine treatment agreed with those reported by El-Tohamy *et al.* (2012) who found that the higher level of Putrescine significantly increased yield of Cape gooseberry. Putrescine up to 100 mg L⁻¹ significantly increased the chickpea yield criteria (number of branches, pods and seeds/ plant, seed and straw yield/ plant and/ fed and biological yield/ fed (Amin *et al.*, 2013).

Increase of grain yield in response to 20 mgL⁻¹ paclobutrazol may be attributed to an increase in number of tillers and spikes. Recently, Youssef and Abd El-Aal, (2013) mentioned that PP333 treatments on *tabernoemontana coronaria* stapf plant resulted in increasing the thickness of lamina (leaf blade) and palisade tissue and that are of great interest to create more chloroplasts to improve the many formed flowers characteristics. The obtained results of yield regarding paclobutrazol are in agreement with Mekki and El-Kholy, (1999); Orabi, (2004); Bekheta and Orabi *et al.*, (2010).

Table 2: Effect of foliar treatment of spermine and paclobutrazol on yield traits of two cultivars of wheat. (Data are means of two seasons).

Cultivar	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	
Treatments	Spikes No/plant		Spikes wt/plant (g)		Grains wt/plant (g)		Grains No/plant		Grains No/spike		1000 Grain wt (g)		
Control	4.00 ^{cd}	4.55 ^{bc}	5.61 ^{cde}	6.04 ^{abc}	3.53 ^{cd}	4.15 ^{bcd}	83.11 ^{de}	96.11 ^{cd}	20.79 ^e	21.12 ^{de}	42.41 ^{bc}	43.19 ^{abc}	
Spermine	20	4.22 ^{bc}	4.89 ^{abc}	6.16 ^{abcde}	6.48 ^{ab}	4.07 ^{bc}	5.30 ^b	94.44 ^{cd}	116.22 ^b	22.40 ^{bcd}	23.76 ^{ab}	43.03 ^{abc}	45.64 ^{ab}
	40	4.55 ^{abc}	5.44 ^a	6.78 ^{ab}	7.53 ^a	4.73 ^b	6.26 ^a	103.67 ^{bc}	136.33 ^a	22.72 ^{bc}	25.04 ^a	45.84 ^a	45.89 ^a
Paclobutrazol	20	4.00 ^{cd}	4.78 ^{ab}	5.75 ^{bcdde}	6.31 ^{de}	3.80 ^{bcd}	5.11 ^b	90.00 ^d	115.55 ^b	22.53 ^{bcd}	24.18 ^a	42.26 ^c	44.24 ^{abc}
	40	3.22 ^d	4.11 ^{bc}	3.91 ^f	5.12 ^c	2.90 ^d	3.55 ^{bc}	69.55 ^e	85.89 ^{cd}	21.56 ^{cde}	20.96 ^{bcd}	41.94 ^c	41.31 ^c

Effect on biochemical traits:

Fig (1 and 2) showed the effect of spermine and PP333 on total phenol and PAL enzyme activity. Spermine treatment (20 and 40 mgL⁻¹) significantly increased total phenol and PAL enzyme activity in fresh leaves of the two studied cultivars especially cv. Gimeza 9 rather than at Giza 168. The obtained results are in a good harmony with those of Abd El Wahed and Gamal El Din (2004) who mentioned that the polyphenol (spermidine) treatment (25, 50 and 75 mgL⁻¹) significantly increased phenolic content of chamomile leaves compared with control. Additionally, spermidine treatments on radish plant under stress resulted in increments in total phenolic compounds (Choudhary *et al.*, 2012). It might be due to the conversion of polyphenol into content in plant. Whereas, it could be derived to sugars, free amino acids, phenolic compounds and essential oil (Herman, 1976).

PP333 effects on total phenol Fig. (1) resulted in nonsignificant increments in total phenols as compared to the corresponding controls. In this respect, Rademacher, 2000 reported that PP333 reduces plant growth without directly interfering with secondary metabolite biosynthetic pathways as it inhibits gibberellin synthesis down stream in the chain of reactions leading to the production of secondary metabolites i.e the biosynthesis of secondary of tannins, phenolic compounds and terpenoids. However, Charbadjan *et al.* (2011) found that PP333 treatments had no effect on phenol but it increase tannins in paper birch and Australian pine. Concerning PAL enzyme (phenylalanine ammonia-lyase) activity, spermine at 20, 40 mgL⁻¹ followed by 20 mgL⁻¹ PP333 recorded significant increments in this enzyme which indirectly improves plant resistance to environmental stresses through production of phenol. Where it is considered to be the principal enzyme of the phenylpropanoid pathway, catalyzing the transformation by deamination of l-phenylalanine into trans-cinnamic acid, which is the prime intermediary in the biosynthesis of phenolics (Levine *et al.*, 1994).

Effect on biochemical constituents of the yielded grains:

The results in table (3) showed the effect of different concentrations of spermine and paclobutrazol (20 and 40 mgL⁻¹) on carbohydrate constituents (glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates) of grains of the two used wheat cultivars. Data clearly show that, spermine with different concentrations increased significantly all carbohydrate constituents of the two cultivars namely Giza 168 and Gimeza 9. Data also, show that, increasing spermine concentrations increased gradually carbohydrate constituents of the yielded grains. Regarding to paclobutrazol, foliar treatment with low concentration (20 mgL⁻¹) caused significant increases in the studied carbohydrate constituents of the yielded wheat grains of the two wheat cultivars plant as compared with control plants. Meanwhile higher concentrations of paclobutrazol (40 mgL⁻¹) decreased all carbohydrate constituents of the yielded wheat grains. Table (3) also shows the superiority of cultivar Gimeza 9 over cultivar Giza 168 of carbohydrates constituents of the yielded grains. These increments were closely correlated to the stimulation of leaves number and dry weight of shoot (Table 1). These obtained data of polyamine treatment agreed with those obtained by Brenner (1988) who proved that, polyamines promote growth influenced the level of phytohormones to play an important role in controlling the sink function

during fruit filling of tomato. Talaat *et al.*, (2005) stated that putrescine treatment increased total soluble sugars, total insoluble sugars and total protein of *Catharanthus roseus*. Hozayn *et al.*, (2008) found that putrescine treatment increased carbohydrate contents of faba bean plant. Alsokari (2011) stated that, spermine treatment increased total soluble sugars, polysaccharides and carbohydrates contents of *Vigna sinensis*. Additionally, Li *et al.*, (2014), under water stress found that white clover plants treated with spermidine exhibited more accumulated organic solutes including soluble sugar, reducing sugar, betaine and free proline. These obtained increases may be attributed to the role of polyamines in increasing photoassimilates and subsequently the growth rate of wheat plant. With respect to paclobutrazol, the obtained results go on line with that obtained by Saker (2004) on *Hibiscus rosa sinensis* and *Tabernaemontana coronaria* shrubs, and Jungklang and Saengnil (2012) on patumma and Youssef and Abd El-Aal. (2013) confirmed our obtained results using paclobutrazol on *Tabernaemontana coronaria* plant. They stated that, as for the explanation of the incremental effect of paclobutrazol on carbohydrate contents, it could be illustrated here on the basis that paclobutrazol treatments stimulated the endogenous cytokinins and there is an intimate relationship between cytokinins and chlorophylls metabolism i.e., cytokinins retard chlorophylls degradation, preserve it and increase its synthesis and this stimulate the output of chlorophyll (Devlin and Witham, 1983).

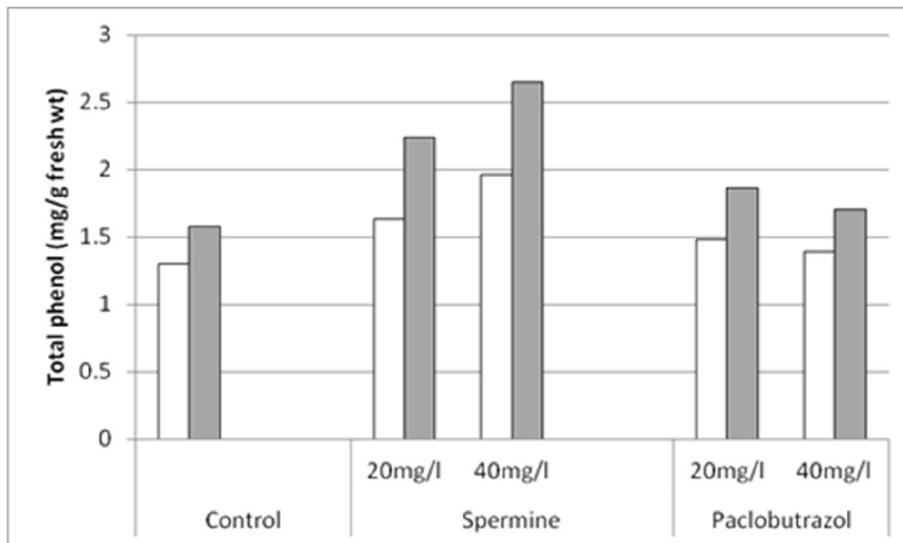


Fig. 1: Effect of foliar treatment of spermine and paclobutrazol on total phenol content (mg/g fresh wt) of leaves of two cultivars of wheat (white bars are Giza 168 cultivar and grey bar are Gimeza 9 cultivar).

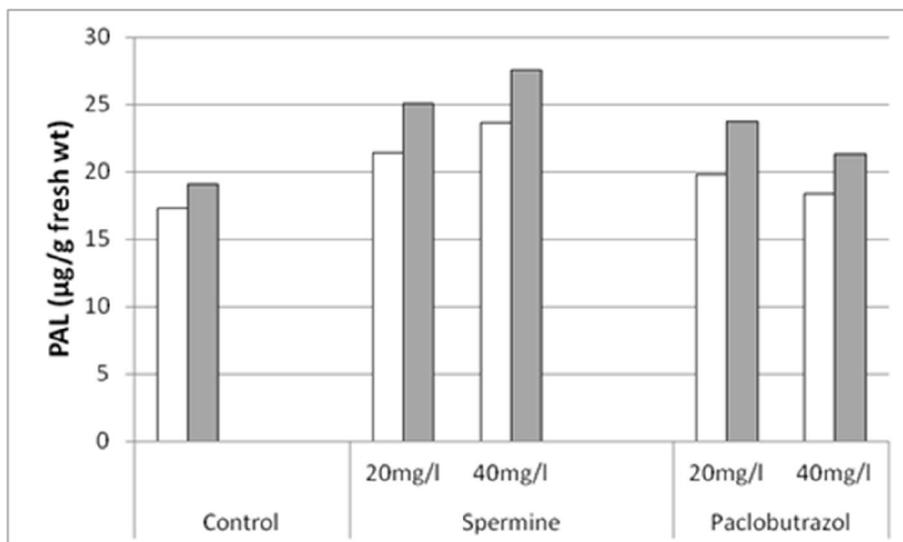


Fig. 2: Effect of foliar treatment of spermine and paclobutrazol on PAL enzyme activity (µg/g fresh wt) of leaves of two cultivars of wheat (white bars are Giza 168 cultivar and grey bar are Gimeza 9 cultivar).

Table 3: Effect of foliar treatment of spermine and paclobutrazol on carbohydrates content (mg g⁻¹ d wt) of yielded grains of two cultivars of wheat plants.

Cultivar	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9
Treatments	Glucose		Sucrose		TSS		Polysaccharides		Total carbohydrates	
Control	0.48 ^e	0.65 ^{cd}	11.65 ^e	13.82 ^d	15.35 ^{de}	17.62 ^{bc}	478.15 ^{cd}	474.38 ^{de}	495.83 ^{cde}	484.63 ^e
Spermine	20	0.68 ^c	0.88 ^a	17.30 ^c	19.56 ^{ab}	17.08 ^{cd}	19.65 ^a	509.85 ^{ab}	494.51 ^{bc}	522.07 ^{ab}
	40	0.77 ^b	0.87 ^a	18.33 ^{bc}	19.92 ^a	17.13 ^{cd}	19.99 ^a	525.85 ^a	507.84 ^{ab}	540.60 ^a
Paclobutrazol	20	0.59 ^d	0.71 ^{bc}	14.53 ^d	18.57 ^{abc}	15.55 ^{de}	18.18 ^{abc}	487.71 ^{cd}	482.40 ^{cde}	499.83 ^{cde}
	40	0.45 ^e	0.59 ^d	10.89 ^e	12.08 ^e	14.68 ^e	16.83 ^{cd}	477.15 ^{cde}	464.84 ^e	492.50 ^{de}

The data recorded in Table (4) indicate clearly that amino N, total soluble N, proline, protein N, total N and total protein gave a stimulatory response as a result of foliar treatment of spermine treatment of the two tested wheat cultivars. Paclobutrazol treatment on two wheat cultivars with low concentrations increased significantly in most cases the studied nitrogenous constituents of the yielded wheat grains of the two cultivars, meanwhile high concentration decreased nitrogenous constituents of the yielded grains as compared with the corresponding controls. The obtained results of spermine are in accordance with findings reported by Zeid (2004) and Abd El- Monem (2007) who mentioned that, polyamine putrescine application resulted in increased in the synthesis of macromolecules particularly protein and photosynthetic pigments of bean and wheat plants, respectively. Polyamines have been found to affect protein synthesis and nitrogenous compounds metabolism as mentioned by Aldesuquy *et al.*, (2013) who showed that spermine treatment on wheat plant increased nitrogenous constituents. Polyamines effects may be attributed to their binding to negatively charged macromolecules (Messiaen *et al.*, 1997). The increases in nitrogenous constituents in response to spermine treatment on wheat plant clearly indicated that the increase in nitrogenous constituents of the yield grains may be due to increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthates supply to grains (Kaur-Sawhney *et al.*, 1982). Regarding to paclobutrazol effect, Ibrahim *et al.*, (2007) found that, total seed proteins contents were evaluated in dry faba bean seeds in response to paclobutrazol treatment. Nouriyani *et al.*, (2012) found that, low level of paclobutrazol treatment decreased soluble proteins of wheat plant.

Table 4: Effect of foliar treatment of spermine and paclobutrazol on nitrogenous constituents (mg g⁻¹ d wt) in yielded grains of two cultivars of wheat.

Cultivar	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9	Giza 168	Gimeza 9
Treatments	Amino N		Total soluble N		Proline		Total - N		Protein N		Total protein	
Control	2.93 ^f	4.67 ^{bc}	18.28 ^f	23.28 ^e	0.72 ^e	0.87 ^e	25.18 ^g	31.93 ^e	6.90 ^{fg}	8.65 ^d	139.10 ^k	147.97 ⁱ
Spermine	20	3.87 ^d	4.93 ^b	26.48 ^d	32.04 ^b	0.91 ^{bc}	0.98 ^{ab}	34.68 ^d	43.92 ^b	8.20 ^{de}	11.88 ^b	157.03 ^c
	40	4.03 ^d	5.61 ^a	29.17 ^c	34.98 ^a	0.96 ^b	1.04 ^a	38.91 ^c	48.21 ^a	9.74 ^c	13.23 ^a	161.67 ^c
Paclobutrazol	20	3.45 ^e	4.84 ^{bc}	21.97 ^e	29.85 ^c	0.78 ^{de}	0.91 ^{bc}	29.27 ^f	40.08 ^c	7.30 ^{ef}	10.23 ^c	146.93 ^g
	40	2.71 ^f	4.59 ^c	16.00 ^g	22.27 ^e	0.71 ^e	0.84 ^{cd}	22.12 ^h	30.13 ^f	6.12 ^g	7.86 ^{def}	136.33 ^j

Conclusion:

Spermin might ascertain its regulatory functions in plant tolerance and paclobutrazol might ascertain its lodging resistance, where the two growth regulators might focused different roles for improvement of productivity and quality of the two studied wheat cultivars especially the more responsive cultivar Gimeza 9.

References

- Abd El Wahed, M.S.A and K.M. Gamal El Din, 2004. Stimulation of growth, flowering and biochemical constituents of chamomile plant (*Chamomilla recutita* L., Rausch) with spermidine and stigmasterol application. Egypt. J. Hort. 31: 45-58.
- Abd El-Monem, Amany A., 2007. Polyamines as modulators of wheat growth, metabolism and reproductive development under high temperature stress. Ph.D. Thesis, Ain Shamas Univ., Cairo, Egypt.
- Al Cazar, R., T. Altabella, F. Marco, C. Bortolotti and M. Reymond, 2010. Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. *Planta*, 231: 1237-1249.
- Al-Abdul Kreem, S.S., 1993. Physiological studies on the effect of uniconazole on wheat, Ph. D. Thesis. College of Science for girls, Dammam. KSA.

- Aldesuquy, H. S., A. Z. Baka and B. M. Micky, 2013. Does exogenous application of kinetin and spermine mitigate the effect of sea water on yield attributes and biochemical aspects of grains?. J. Stress Physiol & Biochem., 9(2): 21-34.
- Alsokari, S. S., 2011. Synergistic effect of kinetin and spermine on some physiological aspects of seawater stressed *Vigna sinensis* plants. Saudi Journal of Biological Sciences, 18: 37-44
- Amin, A.A., F.A. Gharib, H.F. Abouzienna and M.G. Dawood, 2013. Role of Indole – 3 – butyric and or/ and putrescine in Improving productivity of chickpea (*Cicer arietinum* L.). Plants. Pakistan J. of Biol. Sci. 16 (24): 1894-1903.
- Ayad, H.S, F. Reda and M.S.A. Abdalla, 2010. Effect of putrescine and zinc on vegetative growth, photosynthetic pigments, lipid peroxidation and essential oil content of Geranium (*Pelargonium gravealens* L.) world J. of Agric. Sci. 6(5): 601-608.
- Beaudoin – Egan, L. and T. Thorpe, 1985. Tyrosine and phenylalanine ammonialyase activities during shoots inhibition in tobacco callus cultures. Plant Physiol. 78: 348-441.
- Bekheta, M.A., A. H. EL – Ghorab and M.H.S. Mahgoub, 2003. Influence of stigmaterol and uniconazole on growth, endogenous hormones, chemical compositions and radical scavenging activity of thyme (*Thymus serpyllum* L.) essential oil. Egypt. J. Agric. Res. NRC., Cairo, Egypt, 1: 523-545.
- Bekheta, M. A. and I. M. Talaat, 2009. Physiological response of Mung bean "*Vigna radiata*" plants to some bioregulators. J. App. Botany and Food Quality, 83:76-84.
- Berova, M.Z. Zlatev, N. Stoeva, 2002. Effect of paclobutrazol on wheat seedlings under low temperature stress. Bulg. J. Plant. Physiol., 28 (1-2): 75-84.
- Brenner M. L., 1988. The role of hormones in photosynthate partitioning and seed filling In: Plant hormones and their role in plant growth and development 474 – 493 P. J. Davies ed. Kluwer Academic Pub. And Dordrecht, the Netherlands.
- Charbadjan, R. A., P. B. Bonella and D. A. Herms, 2011. Effect of the growth regulator paclobutrazol and fertilization on defensive chemistry and herbivore resistance on Australian pine (*Pinus nigra*) and paper Birch (*Belula papyrifera*). Arboriculture & Urban Forestry. 37(6): 278- 287.
- Chinbal, A.C., M. W. Rees and E. F. Williams, 1943. The total nitrogen content of egg albumin and other proteins. Biochem. J., 37: 354- 357.
- Choudhary, S.P., H.V. Oral, R. Bhardwaj, J. Yu and L. P. Tran, 2012. Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus satius*. J. Exp. Bot. 63 (15): 5659-5675.
- Currey, C. J. and R. G. Lopez, 2010. paclobutrazol pre – plant bulb dips effectively control height of 'Nellie white' easterlily. Horticultural technology 20 (2): 357-360.
- Davis, P.J., 1995. Plant hormones: physiology and biochemistry and biology P. 159 Kluwer academic publishers, London.
- Davis, T.D. and A.S. Andersen, 1998. Growth retardants as aids in adapting new floricultural crops to pot culture. Acta Horticulture, 252: 77-85.
- Devlin, M. and H. Witham, 1983. Plant Physiology, 4th Ed. Publishers Willard, Grant Press, Boston.
- El-Bassiouny, H.M., H.A., Mostafa, S.A. EL- Khawas, R.A. Hassanein, S.I. Khalil and A.A. Abd El-Monem, 2008. Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Aust. J. Basic and Ap. Sci., 2(4): 1390-1403.
- El-Tohamy, W.A., H.M. EL-Abagy, M.A. Badr, A.A. Ghoname and S.D. Abou–Hussein, 2012. Improvement of productivity and quality of cape gooseberry (*Physalis peruvianal.*) by foliar application of some chemical substances. J. Applied Sci. Res. 8: 2366-2370.
- Evans, T.p and R.L. Malmberg, 1989. Do polyamines have roles in plant development? Annu. Rev. Plant Physiol. Plant. Mol. Biol., 40: 235.
- Feteris, A.W., 1965. A serum glucose method without protein precipitation. American J. Medical Technol., 31: 17- 21.
- Fletcher, R.A., A. Gill, T.D. Davis and N.S Ankhla, 2000. Triazoles as plant growth regulators and stress protestants. Horticulture Review, 24: 55-138.
- Galston, A.W. and R. Kaur – Sawhney, 1995. Polyamines as endogenous growth regulators. In Plant Hormones. Physiology, Biochemistry and Molecular Biology, Edited by Davies, P.J. PP. 158-178. Kluwer Academic Publishers, Dordrecht.
- Gomez, K.A. and A.A. Gomez, 1984.. Statistical Procedures for Agricultural Research. John Wiley & Sons Inc., Singapore, 680.
- Handel, E.V., 1968. Direct microdeterminations of sucrose. Analytical Biochem, 22: 280- 283.
- Herbert, D., P.J. Phipps and R.E. Strange, 1971. Chemical analysis of microbial cells. Methods in Microbiology 5B: 209 -344.
- Herrman, K., 1976. Flavonoids and flavones in food plants, J. Food. Technol., 11, 433.

- Hozayn, M., Mona G. Dawood and Mervat Sh. Sadak, 2008. Physiological effect of arginine or putrescine on growth, yield and chemical constituents of faba bean (*Vicia faba* L.) grown in newly reclaimed sandy soil. J. Agric. Sci. Mansoura Univ. 33(3): 1771-1781.
- Ibrahim, I.S., A.B. Abdel-Razik and M. Ebeed Naglaa, 2007. Effect of some plant growth regulators on biochemical gene expression, growth and yield of faba bean. Pak. J. Biotechnol. 4 (1-2): 47-64
- Imai, K., T. Matsuyama, Y. Hanzawa, T.A. Kiyama, M. Tamaoki, H. Saji, Y. Shirano and T. Takahashi, 2004. Spermidine synthesis genes are essential for survival of Arabidopsis. Plant Physiol., 135: 1565-1573.
- Jung, J. and W. Rademacher, 1983. In plant growth regulating chemicals – cereal grains. 254-266, L.G. Nichell, ed. CRC Press.
- Jungklang, J. and K. Saengnil, 2012. Effect of paclobutrazol on patumma cv. Chiang Mai Pink under water stress. Songklanakarin J. Sci. Technol., 34(4): 361-366.
- Kaur-Sawhney, R., H. E. Shih-Flores and A. W. Galston, 1982. Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol., 69: 405 – 410.
- Khan, H.A., K. Ziaf, M. Amjad and Q. Iqbal, 2012. Exogenous application of polyamines improves germination and early seedling growth of hot pepper. Chilean J. Agric. Res., 72: 429-433.
- Kraus, T.E., R.C. Evans., R.A. Fletcher, K.P. Pauls, 1995. Paclobutrazol enhances tolerance to increased levels of UV-B radiation in soybean (*Glycine max*) seedlings. Can. J. Bot 73: 797-806.
- Kuznetsov, V., N.L. Radyukina and N.I. Shevyakova, 2006. Polyamines and stress biological role, metabolism and regulation. Russian J. Plant Physiol., 53: 583-604.
- Lecain, D.R., K.A. Schekel and R.L. Wample, 1986. Growth retarding effects of paclobutrazol on weeping fig. Hort. Sci., 21: 1150-1152.
- Levine, A., R. Tenhaken, R. Dixon, C. Lamb, 1994. H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell. 79:583-593.
- Liu, J.H, C. Honda and T. Moriguchi, 2006. Involvement of polyamine in floral and fruit development. Jpn. Agric. Res. Q., 40: 51-58.
- Liu; K., H.H. FU, Q.X. Bei and S. Luan, 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiol., 124: 1315 – 1325.
- Li, Z, Y. Peng, X.-Q. Zhang, M.-H. Pan, X. Ma, L.-K. Huang and Y.-H. Yan (2014). Exogenous spermidine improves water stress tolerance of white clover (*Trifolium repens* L.) involved in antioxidant defence, gene expression and proline metabolism. POJ., 7(6): 517-526.
- Lurie, S., R.Z. Lipsker, B. Aloni, 1994. Effects of paclobutrazol and chilling temperatures on lipids, antioxidants and ATPase activity of plasma membrane isolated from green bell pepper fruits. Physiol. Plant., 91 : 593-598.
- MSTAT-C., 1988. MSTAT-C, a microcomputer program for the design, arrangement and analysis of agronomic research. Michigan State University, East Lansing.
- Mansuroglu, S., O. Karaguzel, V. Ortacesme and M.S. Sayan, 2009. Effect of paclobutrazol on flowering, leaf and flower colour of *Consolida orientalis*. Pakistan J. Botany, 41 (5): 2323-2332.
- Mekki, B.B and S.A. Orabi, 2007. Response of prickly oil lettuce (*Lactuca scariola* L.) to uniconazol and irrigation with diluted sea water. American – Eurasian J. Agric & Environ. Sci., 2(6): 611-618.
- Mekki, B.B. and M.A. El-Kholy, 1999. Response of yield, oil and fatty acid contents in some oil seed rape varieties to mepiquat chloride. Bull. N.R.C., Egypt, 24: 287-299.
- Messiaen, J., P. Campier and P. van Cutsem, 1997. Polyamines and pectins. I. Ion exchange and selectivity. Plant Physiol., 113: 387-95
- Miller, L. and J.A. Houghton, 1945. The micro-kjeldahl determination of the nitrogen content of amino acids and proteins. Biological Chemistry, 159: 373-383.
- Muting, D. and E. Kaiser, 1963. Spectrophotometric method of determination of α - amino-N in biological material by means of the ninhydrin reaction. Hoppe Seylers Z. Physiol. Chem., 332: 276- 289.
- Nagarajan S. and J. Rana, 2002. Physiological traits associated with yield performance of spring wheat (*Triticum aestivum*) under late sown condition. Indian J. Agric. Sci., 72: 135-140
- Nouriyani, H., E. Majidi, S.M. Seyyednejad, S.A. Siadat and A. Naderi, 2012. Effect of paclobutrazol under different levels of nitrogen on some physiological traits of two wheat cultivars (*Triticum aestivum* L.). World Applied Sciences Journal 16 (1): 01-06,
- Orabi, S.A. 1994. Studies On the influence of Mepiquat chloride on physiological activities and yield of wheat. M.Sc. thesis. Ain Shams Univ.
- Orabi, S.A., 2004. Physiological impacts of cold injury on cucumber (*Cucumis sativus* L.). Plant Ph.D. Thesis, Fac. Sci. Cairo Univ.
- Orabi, S.A., S.R. Salman and M.A.F. Shalaby, 2010. Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. World J. Agric. Sci., 6(3): 252-259.
- PGRSA, 2007. Plant growth regulation hand book of the plant growth regulation society of America 4th Edition.

- The plant growth regulation society of America, Athens.
- Pang, X.M., Z.Y. Zhang, X.P. Wen, Y. Ban and T. Moriguchi, 2007. Polyamines all-purpose players in response to environment stresses in plants. *Plant Stress J.*, 1 : 173-188.
- Paschalidis, K.A. and K.A. Roubelakis – Angelakis, 2005. Sites and regulation of polyamine catabolism in the tobacco plant. Correlations with cell division / expansion, cell cycle progression and vascular development. *Plant Physiol.*, 138: 2174-2184.
- Pegg, A.E., 1986. Recent advances in the biochemistry of polyamines in eu Karyotes. *Biochem. J.*, 234 : 249-62.
- Pinhero. R.G, R.A. Fletcher, 1994. Pacllobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. *Plant Growth Reg.*, 15 : 47-53.
- Pirie, N.W., 1955. Proteins. In: *Modern methods of plant analysis.* (K Peack and M V Tracey, eds, IV, 23, Springer Verlage, Berlin.
- Rademachen, W., 2000. Growth retardants effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Physiology and Plant Molecular Biology.*, 51:501-31.
- Ribeiro, D.M., M. Caroline, B. Jakson, B.R. Glayton and R.S. Barros, 2011. Effects of autoclaving on the physiological action of pacllobutrazol. *Agric. Sci*, 2(3): 191-197.
- Sadak, Mervat Sh., Abd El-Monem, A.A. El-Bassiouny, H.M.S. and Nadia M. Badr, 2012. Physiological response of sunflower (*Helianthus annuus* L.) to exogenous arginine and putrescine treatments under salinity stress. *Journal of Applied Sciences Research*, 8(10): 4943-4957
- Saker, A.M.I., 2004. Physiological studies on growth and flowering of some ornamental shrubs. M.Sc. Thesis Fac. Agric. Moshtohor, Zagazig Univ.
- Schuber, F., 1989. Influence of polyamines on membrane functions. *Biochem. J.*, 260 : 1-10.
- Singh S. and M. Pal, 2003. Growth, yield and phenological response of wheat cultivars to delayed sowing. *J. Plant Physiol.*, 8,(3): 277-286.
- Singleton, V.L, R. Orthofer and L. Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin – Ciocalteu reagent. In: L. Packer, (Ed) *Methods in Enzymology, Oxidants and Antioxidants.* Academic press, PP: 152-178.
- Smith, T.A., 1985. Polyamines. *Annu. Rev. Plant Physiol.*, 36: 117-143.
- Taha, L.S., H.A.A. Taie, M.M. Hussein, 2015. Antioxidant properties, Secondary metabolites and Growth as affected by application of putrescine and Moringa leaves extract on Jojoba plants, *J.APP Pharmaceutical Sci.*, 5(01):030-036.
- Taiz, L. and E. Zeiger, 2006. *Plant physiology.* 4th edition. Sinauer Associates, Inc., Publishers, sunderland.
- Talaat, I. M., M. E. Bekheta and M.H. Maghgoub. 2005. Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. *Int. J. Agric. Biol.*, 7(2):210-213.
- Thayermanavan V, Sadasivam S, 1984. Quall Plant Foods *Hum Nutr* 34,253–257. Quoted from *Biochemical Methods*, Sadasivam S, and A. Manickam, eds) 2nd ed. 11 – 12. New ag. inter. Limit. Publ. New Delhi, India
- Yamaji, H., N. Katsura, T. Nishijima and M. Koshioka, 1991. Effect of soil applied uniconazole and prohexadione calcium on the growth and endogenous gibberellins content of *Lycopersicon esculentum* Mill seedlings. *J. Plant Physiol.*, 138: 763-764.
- Yemm E.W. and A.J. Willis, 1954. The estimation of carbohydrates by anthrone. *Biochem. J.*, 57: 508- 514.
- Yemm, E.W. and A. J. Willis, (1956): The respiration of barely plants. IX. The metabolism of roots during the assimilation of nitrogen. *New Phytol.*, 55: 229- 252.
- Youssef, A.A., M. S. Aly, E.N abou Zeid, L. Iliey and S. Titiana, 2002. Effect of some growth substances on mass production and volatile oil yield of *Mentha piperita* E. "Bulgaro" Egypt. *J. App. Sci.*, 176 (11): 610-623.
- Youssef, A.S.M and M.M.M. Abd El-Aal, 2013. Effect of pacllobutrazol and cycocel on growth, flowering, chemical composition and histological features of potted *Tabernoemontana coronaria* stapf plant. *J. App. Sci. Res.*, 9 (11): 5953-5963.
- Zeid, I.M., 2004. Response of bean (*Phaseolus vulgaris*) to exogenous putrescine treatment under salinity stress. *Pakistan J. Biol. Sci.*, 7: 219- 225.