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Effect of Paclobutrazol and NAA on Sex Determination and Seed Yield of Medicinal Pumpkin (*Cucurbıta pepo* L.)

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Abstract
In order to evaluate the effect of plant growth regulators on sex determination and yield, an experiment was conducted by spraying different hormonal combinations on medicinal pumpkin. Experimental treatments were included different concentrations of NAA (25, 75, 100, 125 mg/l), paclobutrazol (50, 100, 200 and 300 mg/l) and combination of growth regulators (NAA 25 mg/l + PBZ 50 mg/l), (NAA 75 mg/l + PBZ 100 mg/l), (NAA 100 mg/l + PBZ 200 mg/l), (NAA 125 mg/l + PBZ 300 mg/l), and control (no application of growth regulators). Growth regulators were applied on the plants by spraying at the –two-leaf and four-leaf stages. Results showed that spraying paclobutrazol and NAA caused increase in number of female flowers, fruits per plant, seeds per fruits, 1000 seed weight and seed yield per hectare, but the number of male flowers and ratio of male to female flowers were decreased compared to their values in control plants. The highest fresh and dried seed yield of pumpkin seed was obtained with 50 mg/l paclobutrazol.

Keywords: NAA, Pumpkinseed, Paclobutrazol, Sex expression, Seed yield.

Introduction

Cucurbita pepo is a member of the Cucurbitaceae family. This plant bears both male and female blossoms on the same plant (monoecious) (Lerner, 2003). Male flowers are produced early during development in the axes of nodes, and after several stages of male flower productions the female flowers appear in the following nodes (Pen˜aranda et al., 2007). At the beginning of the 20th century a mutant of *Cucurbita pepo* L. var. styriaca, was introduced and named ‘Pumpkinseed’ or ‘Hull–less pumpkin’ (Teppner, 2000). Pumpkin seeds contain approximately 30-50% oil, composed mainly of fatty acids, tocopherols (β and γ) and carotenoids. Pumpkin seed oil has shown to possess strong antioxidant properties in animal experiments (Stevenson et al., 2007). The results indicated that, pumpkin seeds were effective not only in reducing symptoms associated with benign prostate hyperplasia, especially in its early stages, but also no side effects were reported by the patients involved in the trial (Friederich et al., 2000). The sex expression of summer squash is determined by both genetic and environment (e.g. photoperiod and temperature). Because of high

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temperatures and long photoperiods, summer squash usually shows more male flowers and fewer female flowers. This results in decrease in summer squash fruit yield. Many kinds of plant growth regulators have been previously applied in production of *Cucurbita* crops (Li Shuxuan et al., 1979).

Plant growth regulators (PGRs) especially auxins and gibberellins are involved on developmental processes, seed germination, floral differentiation, flowering and seed biochemical composition in many plants (Al-khassawneh et al., 2006; Jaleel et al., 2007). Low fruit formation due to restricted female flower induction (Stepleton et al., 2000) is the most important problem in pumpkin cultivation. Application of indole-3-acetic acid (IAA) enhances ethylene and ACC production in pumpkin. The stimulatory effect of IAA has been more reported in gynoecious species (Trebitsh, et al., 1987). Ethylene is a plant growth regulator known to alter sex expression in plants belonging to the Cucurbitaceae family. It increases the number of pistillate flowers when applied to monoecious plants (Papadopoulou et al., 2005; Thappa et al., 2011). Fruit yield of medicinal pumpkin is low due to restricted induction of female flowers and weakness of fruit set. It has been reported that NAA is effective in inhibiting induction of male flowers and increasing the number of female flowers (Ntui et al., 2007). Sedghi et al., (2008) showed that fruit yield would be promoted with NAA application. This increase is probably related to induction of female flowers per plant.

Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellins synthesis (Kamountsis et al., 1999). Paclobutrazol (PBZ) through inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid via inactivating cytochrome P450-dependent oxygenases is a potent inhibitor of gibberellins biosynthesis (Graebe,. 1987). Sedghi et al., (2008) showed that GA_3 spraying increased number of male flowers. Paclobutrazol reduces plant growth without directly interfering with secondary metabolite biosynthetic pathways, as it inhibits gibberellin synthesis downstream in the chain of reactions inducing the production of secondary metabolites, i.e., the biosynthesis of tannins, phenolic compounds, and terpenoids (Rademacher, 2000) without negative effects on photosynthesis (Abdollahi et al., 2012; Watson and Jacobs, 2012; Sedighi et al., 2008; Ghosh et al., 2010; Babadaei Samani, 2014). Therefore, objective of present study was to investigate the effect of paclobutrazol and NAA on sex determination and yield of medicinal pumpkin.

Materials and methods

Location of experiment

In order to evaluate the effect of plant growth regulator on medicinal pumpkin, an experiment was conducted during the 2014 growing season in an experimental field in ACECR Kermanshah Higher Education Institute, located on latitude 46° 94´ N and longitude 34° 54´ E in Iran,.

Treatments and growth condition

Pumpkin dry seeds were imbibed overnight at 30˚C before planting. Seeds were sown on 5th of May, 2014 on prepared beds. Each plot had four rows and spacing between-row and between-plant were 200 and 40 cm, respectively. Five seeds per hole were placed at 3 cm planting depth. After germination, seedlings were thinned to one per hole and weeding was regularly carried out. Growth regulators were applied on two times at the two-leaf and four-leaf stages. Control plants were sprayed with distilled water. The spraying was done with a hand sprayer very early in the morning.

Experimental treatments were different concentrations of naphthalene acetic acid (NAA) (25, 75, 100, 125 mg/l), various concentrations of Paclobutrazol (PBZ) (50, 100, 200 and 300 mg/l) and combination of growth regulators (NAA25 mg/l + PBZ 50 mg/l), (NAA75 mg/l + PBZ 100 mg/l), (NAA100 mg/l + PBZ 200 mg/l), (NAA125 mg/l + PBZ 300 mg/l), and control (lack of growth regulators). Randomized complete block design with three replications was used for statistical design of the experiment. The soil was clay loam in texture. After preparing the seed bed, basic fertilizer (nitrogen, phosphorus and potassium) was added based on the results of soil analysis.

Measurements

Fruits were harvested at full maturity when their colour was yellow-orange. Fruits were carefully harvested by hand. The fruits were cut open for easy extraction of seeds. The following characteristics were measured. The number of male and female flowers and ratio of male flowers to female flowers was determined on plants. Number of fruits per plant, number of seeds per fruits, 1000 seed weight and seed yield per hectare, were recorded at the end of the experiment.

Statistical analysis

Data related to the sex and yield of seed pumpkin were analysed by ANOVA using SAS (version 9.1) and means were compared using Duncan's multiple range tests at a significance level of 5%.

Results

Number of male flowers per plant

Effect of growth regulators on floral differentiation was significant (Table 1). The number of male flowers in the control treatment (no use of growth regulators), was significantly more than those of growth regulators applications. Control treatment had the highest number of male flowers per plant. Results showed that NAA and paclobutrazol spraying alone had considerable effects on male flower production; as a result the number of male flowers was significantly affected by NAA and paclobutrazol treatments (Table 1). Increase in the concentration of these growth regulators, caused a decrease in the number of male flowers. Application of NAA and PBZ with together reduced the number of male flowers per plant. Strongest effect was observed in NAA125+PBZ300 and NAA100+PBZ200, in which the number of staminate flowers was decreased, when compared to the number of staminate flowers in control plants (Table 1).

Table 1. Effects of foliar application of plant growth regulators (NAA and PBZ) on sex determination and yield of seed pumpkin

Plant growth regulators (mg L^{-1})	Number of male flowers per plant	Number of female flowers per plant	Ratio of male to female flowers	Number of fruits per plant	Number of seed per fruit
Control	15.96^{a}	4.91^{i}	3.31^{a}	0.87^{e}	221.22^{d}
NAA25	8.64^{c}	5.87^{i}	1.47^{b}	1.00^{de}	241.06^{bc}
NAA75	7.08^{ef}	7.77^{h}	0.91^{c}	1.21^{bcd}	248.27^{ab}
NAA100	7.31^{de}	9.51^{g}	0.78^{cde}	1.24^{bcd}	201.07^{e}
NAA125	6.87^{ef}	11.56^{f}	0.60^{efg}	1.42^{b}	191.39^{ef}
PBZ 50	11.07^{b}	13.00^{ef}	0.85^{cd}	1.70^{a}	233.05^{bcd}
PBZ 100	8.85^{c}	13.48^{e}	0.62^{def}	1.25^{bcd}	264.53^{a}
PBZ 200	6.20^{f}	15.23^{d}	0.41^{gh}	1.26^{bcd}	227.72^{cd}
PBZ 300	8.41^{cd}	16.18^{d}	0.52^{fg}	0.97^{de}	231.01^{bcd}
NAA25+PBZ50	7.30^{def}	18.08^{c}	0.40^{gh}	1.07^{cde}	178.67^{f}
NAA75+PBZ100	7.97^{cde}	19.36^{bc}	0.41^{gh}	1.74^{a}	187.11^{ef}
NAA100+PBZ200	4.42^{g}	20.78^{ab}	0.21^{hi}	1.14^{b-e}	249.33^{ab}
NAA125+PBZ300	4.19^{g}	22.06^{a}	0.19^{i}	1.33^{bc}	153.36^{g}

Note: Means having the same letters in each column are not significantly different by Duncan test, $p < 0.05$.

Number of female flowers per plant

Solitary application of paclobotrazol and NAA significantly increased the number of female flowers per plant as compared to their number in control plants. Data in Table 1 showing that different concentrations of Paclobotrazol and NAA tested in this trial had a significant effect on the number of female flowers per plant. Increasing PBZ and NAA concentrations caused increase in the number of female flowers. These results indicated that co-application of NAA+PBZ is more effective in induction of female flower than their solitary applications. The maximum number of female flowers per plant (22.06) was recorded in plants sprayed with NAA125+PBZ300 treatment. Control treatment had the lowest number of female flowers per plant (4.9).

Sex determination

The effect of PGRs on the male to female flower ratios are presented in Table 1. All growth regulator treatments differed significantly for this reproductive trait (Table 1). Ratio of male to female flowers was highest (3.31) in control plants compared to other treatments. All PGRs treatments decreased the male to female flowers ratio. The lowest male to female flowers ratio was recorded in plants treated with NAA125+PBZ300. The ratio of male to female flowers in this treatment was 0.19.

The number of fruits per plant

Table 1 showed that application of plant growth regulators significantly increased number of fruits. Data regarding fruit set revealed significant differences among different growth regulator treatments. Both plant growth regulators had strongest positive effect on fruit set, in which the number of fruits per plant increased. Highest fruit set (1.74) was recorded in NAA75+PBZ100 but had no significant difference with the 50 mg/l PBZ application. Control treatment had the lowest number (0.87) of fruits per plant (Table 1). Application of growth regulators had good effect on growth and yield of field crops. This may be due to the positive correlation of number of fruits/plant with number of female flowers. Plant growth, flowering and yield have been manipulated through different growth regulating substances.

Seed number

Table 1 present the effect of PGR on seed number per fruit. Results showed significant difference among the type and concentration of PGR on seed number per fruit. This study showed that seed number per fruit was increased as concentration of NAA and PBZ increased to a threshold level. When concentration of NAA and PBZ was in excessive amount, resulted decrease in number of seeds per fruit. It seems higher concentrations of NAA and PBZ has a negative effects on seed production in fruits. Therefore, a threshold level of NAA and PBZ is required to obtain the best effect. The number of seed per fruit enhanced as the concentration of PBZ increased from 50 to 100 mg/l. Highest number of seeds per fruit was obtained in plants sprayed with 100 mg/l PBZ. The number of seed per fruit was decreased as the concentration of PBZ increased from 100 to 300 mg/l (Table 1).

Seed weight per fruit

Table 2 shows significant difference among the type and concentration of PGR on seed weight per fruit. Among different treatments, seed weight per fruit was maximum in NAA25+PBZ50 treatment while minimum seed weight per fruit was recorded in plants treated with NAA125+PBZ300 (Table 2).

Table 2. Effect of foliar application of plant growth regulators (NAA and PBZ) on pumpkin seed characteristics.

Plant growth regulators (mg L^{-1})	Seed weight per fruit (gr)	1000-Seed weight (gr)	Seed fresh weight (kg/h)	Seed dry weight (kg/h)
Control	43.16^{bc}	125.5^{ab}	469.70^{d}	283.67^{de}
NAA25	38.73^{cd}	110.1^{bc}	485.10^{d}	251.00^{e}
NAA75	47.67^{bc}	112.2^{bcd}	747.2^{a-d}	275.71^{de}
NAA100	40.64^{bc}	112.97^{bcd}	610.00^{bc}	296.00^{de}
NAA125	40.19^{bcd}	112.9^{bcd}	529.20^{cd}	351.67^{cd}
PBZ 50	46.82^{cd}	124.4^{abc}	1035.30^{a}	437.33^{a}
PBZ 100	50.95^{b}	134.6^{a}	798.00^{a-d}	389.83^{abc}
PBZ 200	45.94^{bc}	104.4^{ed}	729.30^{a-d}	314.67^{cde}
PBZ 300	48.29^{bc}	94.6^{e}	589.70^{bcd}	253.33^{e}
NAA25+PBZ50	65.43^{a}	127.9^{ab}	894.00^{abc}	424.00^{ab}
NAA75+PBZ100	42.37^{bc}	113.3^{bcd}	924.70^{ab}	421.33^{ab}
NAA100+PBZ200	45.48^{bc}	111.4^{b-e}	760.30^{a-d}	330.33^{bcd}
NAA125+PBZ300	31.32^{d}	107^{cde}	529.20^{cd}	251.67^{e}

Note: Means having the same letters in each column are not significantly different by Duncan test, $p < 0.05$.

Fresh and dried seed yield

Results showed that kind and concentration of PGR had a significant effect on seed yield (Table 2.). The highest Fresh and dried seed yield of pumpkin seed was obtained with 50 mg/l PBZ.

1000-Seed weight

The effect of PGRs on 1000-seed weight is presented in Table 2. There was a considerable difference among growth regulator treatments for this trait. The highest 1000-seed weight of pumpkin seed was obtained with 100 mg/l PBZ while minimum 1000-seed weight was recorded at 300 mg/l PBZ treatment (Table 2). These results indicated that application of NAA had not any significant effect on 1000-seed weight.

Discussion

In the present study NAA and PBZ spraying and especially their co-application reduced the number of male flowers per plant. Many kinds of plant growth regulators have been used on Cucurbitaceous crops (Li Shuxuan et al., 1979). There is crosstalk between the ethylene and other hormone signalling pathways, particularly with auxin, whose effects are often mediated by ethylene (O'Donnell et al., 1996, 2003). Ethylene is a plant growth regulator known to alter sex expression in plants that belonging to the Cucurbitaceae family, while when applied to monoecious plants induce generation of pistillate flowers (Papadopoulou et al., 2005). Previous studies shown that ethephon is effective in controlling expression of female flowers. GA_3 and $AgNO_3$ are effective in controlling the expression of male flowers (Wang Qiaomei et al., 1997; Ying Zhenshi et al., 1990). PBZ is a triazole type plant growth retardant which blocks gibberellin biosynthesis. Triazole compounds are synthetic plant growth regulators that act as anti-gibberellins and known to inhibit generation of male flower in plants (Tafazoli and Beyl, 1993; Pgrsa, 2007; Watson and Jacobs, 2012). Although growth reduction effect of PBZ is common, growth reduction percentage, flowering, leaf area and chlorophyll content, flower shape and colour responses of plants to this chemical can vary depending on the concentration, method of application, site of application, species and cultivar and also growing conditions (Nasr, 1995; Karaguzel, 1999; Banon et al., 2002).

Solitary application of PBZ and NAA significantly increased the number of female flowers per plant as compared to control. A possible reason might be that

PBZ has an anti-gibberellin effect and causes cessation of the mitotic processes in the meristem of shoot, thereby affecting number of female flowers (Hayashi et al., 2001; Ouzounidou et al., 2008). Auxin is known to stimulate greatly both ethylene production and the conversion of methionine to ethylene in vegetative tissues and high concentrations of auxin can induce the synthesis of ethylene. Therefore, high concentration of auxin can induce femaleness of flowers in some species (Yu and Yang, 1979). Girek et al. (2013) showed that Ethrel treatment in melon induced higher number of pistillate flowers but gibberellic acid reduced the number of pistillate flowers per plant. Ntui et al. (2007) investigated the effects of growth regulator (IAA and BAP) applications on the growth characteristics and sex determination in Pumpkin (*Cucurbita ficifolia* L.). Their result showed that the effect of the hormones is dependent on their concentrations and number of female flowers increased in plants treated with IAA 100 mg/l. These results indicated that NAA is effective in female flower induction which is in agreement with the previous reports (Ntui et al., 2007; Mancini and Calabrese, 1999).

PGRs decreased the male to female flowers ratios and male to female flower ratio was highest in control compared to other treatments. This is in line with the results obtained by other researchers (El-Zawily and Arafa, 1981; Ntui et al., 2007). PGRs especially auxins and gibberellins are involved in many plant developmental processes including seed germination, floral differentiation and seed biochemical composition (Al-khassawneh et al., 2006; Jaleel et al., 2007). Ntui et al. (2007) applied four aqueous concentrations of two growth regulators including indole acetic acid (IAA) and benzyl amino purine (BAP) on pumpkin. Number of female flowers significantly increased by IAA spraying in comparison to BAP.

Results of present study showed that application of PBZ and NAA caused an increase in the number of fruits. Low fruit formation due to low female flower induction (Stepleton et al., 2000) is the most important problems in pumpkin cultivation. Hormones regulate physiological process and synthetic growth regulators may enhance growth and development of field crops. NAA can increase fruit setting ratio, prevent fruit dropping and determine flower sex (Raoofi, et al., 2014). Abdollahi et al., (2012) indicated that PBZ reduced vegetative growth by reducing both shoot fresh and dry weights while simultaneously some reproductive characteristics such as inflorescence and fruit number were increased but PBZ reduced fruit weight (primary and secondary fruits). Sedighi et al. (2008) reported that application of PBZ in apricot trees increases the percentage of fruit set. Lolaei et al. (2012, 2013) reported application of PBZ significantly increased number of fruit on plant.

Based on the obtained result, seed number per fruit was influenced by the type and concentration of PGR. Seed number per fruit was increased as concentration of NAA and PBZ increased to a threshold level. Ntui et al (2007) investigated the effects of growth regulator (0, 50, 100 and 150 mg/l IAA and BAP) on the growth characteristics and sex expression in Pumpkin (*Cucurbita ficifolia* L.). Their result showed that the effect of the hormones is dependent on their concentrations. Applications of growth regulators at higher doses (150 mg/l for IAA and 100 and 150 mg/l for BAP) cause a decreasing effect on most of the studied characteristics. Number of seeds per fruit was highest in 50 mg/l IAA.

It has been shown that foliar application of NAA increases plant height, number of leaves per plant, fruit size; as consequence enhancement of seed yield in different crops (Lee, 1990). The increased number of female flowers, fruit set and increased metabolic activity leading to higher translocation of metabolites from source to sink parts which resulted in better development of seed in bitter gourd (Gedam et al., 1998).

Fruit yield of medicinal pumpkin is limited due to lower female flowers and weakness of fruit set. Auxins can increase number of female flowers and induce fruit formation. Therefore, application of auxins is recommended before flowering to achieving high fruit yield in medicinal pumpkin. Sedghi et al. (2008) showed that fruit yield increases by NAA spraying on plants. This increase is probably related to induction of female flowers in plant. PGRs regulate physiological process and synthetic growth regulators may enhance growth and development of field crops thereby increase total dry mass in field crops (Chibu et al., 2000; Dakua, 2002; Rahman, 2004; Islam, 2007; Cho et al., 2008). Enhanced productivity due to PBZ has been earlier reported in several plant species (Senoo and Isoda 2003; Blaikie et al., 2004; Asin et al., 2007).

Conclusion

PGRs are involved on the sex determination in the flower of seed pumpkin. Low fruit formation due to restricted female flower induction is the most important problems in pumpkin cultivation. According to the obtained results, PBZ and NAA can be used to improve yield of pumpkin. The results also indicated that the fresh and dried seed yield of seed pumpkin can be increased by application of 50 mg/l PBZ.

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