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γ-Tocopherol Accumulation and Floral Differentiation of Medicinal Pumpkin (*Cucurbıta pepo* L.) in Response to Plant Growth Regulators

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Abstract

Changes in biochemical and agronomical characteristics were studied in medicinal pumpkin (*Cucurbita pepo* L.) plants under different treatments with plant growth regulators (PGRs). The seeds were subjected to priming with PGRs before planting and plants unerwent foliar sprays with different solutions of PGRs at 10 day-intervals after flowering. γ-tocopherol (γ-toc) content of grains was found to be increased under gibberellic acid (GA3) treatment. The accumulation of γ-tocopherol in the grains of GA3 treated plants was approximately 19.5 % higher than the control and reached 220.2 mg kg-1 dry pumpkin grains. The number of female flowers per plant was positively affected by PGRs and was 10.1 and 1.89 respectively, in the naphthalene acetic acid (NAA) and GA3 treated plants. A significant efficiency of treatments was observed upon fresh fruit yield. The yield increased from 4831 t ha-1 in the control to 6820 t ha-1 with NAA treatment. Priming treatments increased seedling emergence rate and percent. The highest seedling emergence rate was found in GA3 treated seeds.

Keywords: γ-tocopherol, floral differentiation, plant growth regulators, seed priming

Introductıon

Medicinal pumpkin (*Cucurbita pepo* convar. pepo var. styriaca) is an important annual plant that belongs to the *Cucurbitaceae* family. The grains of pumpkin contain medicinal raw materials that are used for producing pharmaceutical products such as peponen, pepostrin and gronfing to overcome prostatic hypertrophy and urinary tract irritation (Horvath and Bedo, 1998; Bremness, 1994). Pumpkin grains contain approximately 30-50% oil, composed mainly of fatty acids, tocopherols (β and γ) and carotenoids (Stevenson et al., 2007). Tocophrols, collectively known as vitamin E, are a class of lipid-soluble antioxidants synthesized exclusively by photosynthetic organisms. Tocopherols are essential components of the human diet because they perform numerous critical functions including quenching and scavenging various reactive oxygen species (ROS) and free radicals and protecting polyunsaturated fatty acids from lipid peroxidation (Murkovic et al., 1996a,b).

Plant growth regulators (PGRs) especially auxins and gibberellins are involved in many plant development processes and alter a number of desirable effects including seed germination, uniformity of germination and flowering, floral differentiation and grain biochemical composition (Alkhassawneh et al., 2006; Jaleel et al., 2007). Emergence and stand establishment of seeds particularly under stress conditions, and low fruit formation due to little female flower induction (Stepleton et al., 2000) are the most important problems in pumpkin cultivation. Seed germination improves in response to various pre-treatments and causes faster and more uniform emergence under a broader range of environmental conditions (Copeland and McDonald, 2001). Seed priming consists of soaking the seeds in different solutions, surface-drying and then planting dry seeds (Murungu et al., 2004; Sparks, 2005). Efficiency of seed priming with PGRs has reported in the literature. Sharma and Saran (1992) demonstrated that soaking blakgram (*Vigna mungo*) seeds in 40 mg/lit gibberellic acid (GA_3) increases germination rate and percentage under non-stress conditions. Increasing seed germination in response to $GA_{4/7}$, benzyl adenine (BA), and daminozide was shown in five bedding plant species (Finch-savage et al., 1991). Gibberellins can induce the production of hydrolytic enzymes responsible to endosperm degradation and cause faster germination (Andreoli and Khan, 1998). Pill and Kilian (2000) showed that matricpriming with polyethylene glycol in combination with GA_3 increases the percentage, rate and synchrony of seedling emergence, and length of hypocotyl at 3 weeks after planting. Staub et al., (1989) used some chemical seed treatment in cucumber and found that $GA_{4/7}$ + Etephon cause a higher percentage of germination than that of all other treatments.

Spraying of pumpkin plants with growth regulators at different growth stages can affect on its floral differentiation. Ntui et al. (2007) applied four aqueous concentrations of two growth regulators contain indole acetic acid (IAA) and benzyl amino purine (BAP) on pumpkin. Number of female flowers significantly increased by IAA spraying in

Table 1 Analysis of variance table for rate and percent of seedling emergence

SOV	df	MS	
		Seedling emergence rate	Emergence percent
Replication	2	118.314 ns	20.313 ns
Priming	2	5044.741 **	453.646 **
Es	27	95.809	7.639
E	4	149.838	9.896
CV (%)		18.69	3.03

ns and ** are non-significant and significant at 1% probability level respectively

comparison to BAP. Also, application of growth regulators at higher doses showed a decreasing effect in most of the characters studied and grain yield was the highest in IAA 50 mg/lit. Foliar spraying of Zucchini plants with ethephon and auxin was reported by Mancini and Calabrese (1999). They applied ethephon treatments to the plants at 3^{rd}-4^{th} true leaf stages where as auxin applications were made when female flowers appeared and then repeated at 7 day-intervals. Results showed that treatments positively affect on plants and number of female flowers increased from 8.6 to 10.2. A significant efficiency of treatment also was observed on yield and earliness. The influence of growth regulators on floral differentiation and yield of *Iris nigricans* Dinsm (Al-khassawneh et al., 2006) jojoba (Prat et al., 2007) and chestnut (Qiguang et al., 1985) was reported.

Lot of works have already been carried out in medicinal pumpkin in its flower differentiation, yield and yield attributes under different fertilizer and pruning treatments (Gholipouri and Nazarnejad, 2007; Aroiee and Omidbaigi, 2004), but the effects of PGRs attracted a little attention in this plant (Mancini and Calabrese, 1999; Ntui et al., 2007) and other cucurbits (El-Zawily and Arafa, 1981; Harlevy and Kader, 1992). To the best of our knowledge, no information on the effect of GA3 and NAA on γ-tocopherol accumulation in this medicinal plant is available. The effects of GA3 and triazoles on antioxidant potentials particularly α-tocopherol and alkaloid production in the vegetative parts of *Catharanthus roseus* were mentioned in the literature (Jaleel et al., 2007 and 2006). This investigation was aimed for finding out the extent of changes in γ-toc, seedling establishment, sex expression and yield in *C. pepo* under priming and spraying of GA3 and NAA treatments.

Materıals and methods

The experiments were carried out in the Babolan Research Station, department of agronomy and plant breeding, University of Mohaghegh Ardabili, Iran, located on latitude 38° 15′ N and longitude 48° 15′ E with annual rainfall mean about 400 mm. Experimental treatments were three priming levels contain control, naphthalene acetic acid (NAA 50 mg lit^{-1}) and GA_3 (2 mg lit^{-1}) with four spraying levels of growth regulators (control, NAA, GA_3 and NAA+GA_3), arranged in a factorial experiment with three replications. Pumpkin seeds were divided to three equal lots before planting, primed with growth regulators and dried after 20 hours priming. Dry seeds were sown on 22 May 2007. Each plot had four rows and spacing between and within the rows were 100 and 40 cm respectively. Five seeds per hole were placed at 3cm planting depth. In order to determining seedling emergence rate, number of appeared seedlings in each plot was recorded daily. Rate of seedling emergence was calculated using a formula proposed by Belecher and Miller (1974). There were four samples in each plot therefore; analysis of variance for this trait was performed as multi-observational randomized complete block design. Growth regulators were applied on the plants by spraying the entire plant before flowering at 10 day-intervals. The spraying was done with a hand sprayer very early in the morning. The number of male and female flowers was determined on plants at each harvest. At full maturity, fruits were harvested when become yellow-orange in color and their fresh weights

Table 2 Analysis of variance table for fresh fruit yield, number of male and female flowers and γ-toc content

SOV	df	MS			
		No. of male flowers	No. of female flowers	Fresh fruit yield	γ-toc
Replication	2	19.299 *	4.771 ns	132837.67 ns	305.088
Priming	2	2.528 ns	1.188 ns	1044184.221 **	1408.998
Growth regulators	3	41.352 **	108.137 **	28293250.364 **	33324.5**
Priming * growth regulators	6	4.046 ns	0.734 ns	2945909.807 **	435.711
Error	22	3.594	1.748	152406.173	580.819
CV (%)		14.71	23.51	8.23	16.23

ns, * and ** are non-significant and significant at 5% and 1% probability level respectively

Table 3 Effect of different seed priming substances on fresh fruit yield, rate and percent of seedling emergence (P<0.01)

Treatment	Emergence percent (%)	Seedling growth rate (seedling day^{-1})	Fresh fruit yield (ton ha^{-1})
Priming with GA3	97.5 a	75.21 a	114.775 b
Priming with NAA	91.04 b	46.36 b	127.025 a
Non-priming	85.21 c	35.55 b	113.8 b

various letters in each column have significant differences at 1% probability level

were recorded. High-Performance Liquid Chromatography (HPLC) was performed to determine the γ-toc content in dry pumpkin grains. The data were subjected to ANOVA analysis, and treatments were compared with Duncan' s multiple range test.

Results and discussion

Effect of different seed priming treatments with PGRs on seedling emergence rate was significant (Table 1). Percent and rate of seedling emergence in GA_3 significantly was greater than the other treatments, while priming with NAA and non-priming treatments had no significant differences in rate of emergence (Table 3). GA_3 induces the production of hydrolytic enzymes responsible to endosperm degradation and causes faster germination (Andreoli and Khan, 1998). In this study, the highest emergence rate was seen in GA_3 treatments about 75.2 seedlings per day. The rate of emergence in NAA was greater than control but, not significant (Table 3). Seed imbibition and initiation of germination activities in NAA solution probably was responsible for greater emergence rate and is not related to NAA regulatory effect. These results are consistent to Finch-Savage et al., 1991; Pill and Kilian, 2000; Staub et al., 1989.

Effect of growth regulators on floral differentiation was significant (Table 2). Results showed that NAA have great effect on female flower production and GA_3 spraying increased number of male flowers (Table 4). These results indicated that NAA is more effective in female flower induction and confirm results of other authors (Ntui et al., 2007; Mancini and Calabrese, 1999; El-Zawily and Arafa, 1981; Harlevy and Kader, 1992). GA_3 and control treatments had no significant difference and both had the highest number of male flowers per plant. The ratio of male to female flowers in NAA spraying was lower than GA_3 (Table 4), indicated that NAA induces formation of female flowers. Therefore, NAA was more effective in inhibiting male flowers and increasing the number of female flowers than GA_3. This confirms results of other authors (El-Zawily and Arafa, 1981; Ntui et al., 2007).

Simple effects of priming and growth regulators and their interaction on fruit yield were significant (Table 2). Seed priming with NAA significantly increased fruit yield while priming with GA_3 and non-priming were not significantly different (Table 3). Seed priming with GA_3 increased seedling emergence rate but, had no effect on fruit yield in comparison with control (Table 3).

Fresh fruit yield under NAA spraying was higher than other treatments (Table 4). This increase is probably related to increasing the number of female flowers per plant. This is consistent with Gholipouri and Nazarnejad (2007) which showed that stem pruning, can increase number of female flowers and consequently increase fruit yield. In this condition, removal of the shoot apex usually results in the growth of one or more of the lateral buds and eliminates the apical dominance. This can probably alter the auxin concentration in the lateral buds and induce female flower production. Therefore, it is concluded that spraying of NAA and stem pruning can replace each other to increasing number of female flowers in pumpkin.

Interaction between seed priming and PGRs application showed that GA_3 priming and NAA spraying have the highest fruit yield (Figure 1). This treatment had no significant difference with NAA priming and its spraying at near flowering with 10 day-intervals. So, achieving the maximum fruit yield in pumpkin is possible by priming and spraying with NAA before flowering.

The γ-toc content of grains in the GA3 treated plants was higher than that of NAA treated and control plants (Table 4). The increase in non-enzymatic antioxidants like α-tocopherol in vegetative organs was reported by Jaleel et al. (2007) under GA3 spraying, but there is no evidence on γ-toc accumulation in pumpkin grains under PGRs

Table 4 Effect of different growth regulators on fresh fruit yield, number of flowers, ratio of male to female flowers and γ-toc content (P<0.01)

Treatment	Fresh fruit yield (ton ha^{-1})	No. of flowers		Ratio (male/female)	γ-toc (mg kg^{-1})
		male	female		
Control	4831 b	13.94 a	4.33 c	3.22	176.9 b
NAA	6820 a	11.33 b	10.11 a	1.12	89.61 c
GA3	2484 c	15.39 a	1.89 d	8.14	220.2 a
NAA+GA_3	4829 b	10.89 b	6.17 b	1.76	107.3 c

various letters in each column have significant differences at 1% probability level

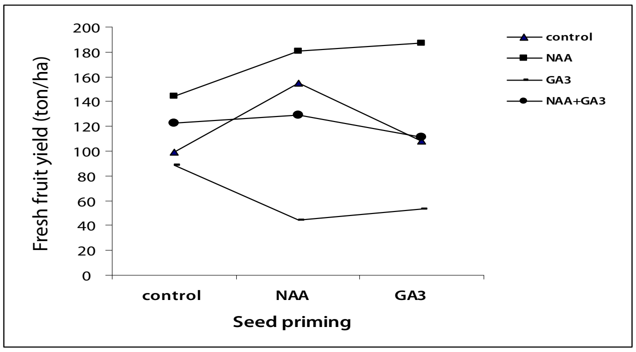

Figure 1 Interaction between seed priming levels and spraying of growth regulators on fresh fruit yield of medicinal pumpkin.

treatment. Jaleel et al., (2007), concluded that exogenous GA3 application at low concentration could be used as a potential tool to increase defense mechanisms and the level of active principles in medicinal plants. PGRs can alter the metabolic equilibrium and exhibit stress like symptoms in plants (Nilsen, 1996). Hildebrand and Grayburn (1991) reported that the increase of ethylene under oxidative stress is associated with lipid peroxidation caused by activated oxygen radicals. Application of auxin can promote ethylene biosynthesis and probably it is responsible for antioxidant reduction in the whole plant.

Conclusions

Fruit yield of medicinal pumpkin is low due to lower female flowers and weakness of fruit set. Auxins can increase number of female flowers and induce fruit formation so, application of auxins is recommended before flowering to achieving high fruit yield in medicinal pumpkin. On the other hand, NAA spraying significantly decreases γ-toc content in the grains. In conclusion, our results indicated that GA3 application at low concentration could be used to increase active ingredients of medicinal pumpkin grains for pharmaceutical consumptions. It should be considered that this approach can decrease fruit and grain yield of this plant per hectare.

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