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EXOGENOUS CYTOKININ APPLICATION INCREASED THE CAPSAICIN AND ASCORBIC ACID CONTENT IN PEPPER FRUIT

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Abstract

Chili peppers synthesize capsaicin and accumulate it in the fruits. Many biochemical studies have been done in the field of capsaicin biosynthesis in chili peppers. The levels of capsaicin vary among chili pepper cultivars, and it is also affected by environmental conditions. The present study assayed the effect of cytokinin on the capsaicin and ascorbic acid content of Capsicum annum var 'Ilica' (pungent pepper) in the greenhouse. Cytokinin levels include 0, 50 and 100 µM of Benzyl amino purine and the treatments were applied at every 10 days until the harvest beginning 5 days after planting. The results of this study monitored that cytokinin (benzyl amino purine) treatment increased the capsaicin and ascorbic acid content in pepper fruits as compared to the control but decreased the fruit number per plant.

***Key words**: ascorbic acid, capsaicin, fruit, pungent pepper.*

INTRODUCTION

Hot pepper burning sensation induced by the presence of acid amides, collectively known as capsaicinoids, that are formed from phenylalanine and valine or leucine. Capsaicin and dihydro indicate more than 90% of the total capsaicinoid content in most pungent peppers (Ben-Chaim et al., 2006). Capsaicinoid biosynthesis is restricted to the genus *Capsicum* (Stewart et al., 2007). Capsaicin content raises slowly during fruit development reaching peak levels at 40 to 50 days after planting (Contreras-Padilla and Yahia, 1998), after which it gravitates to deteriorate into secondary compounds due to peroxidase action (Bernal and Barceló, 1996). For increasing or improving pungent compound production, a research has revealed that hydric stress ameliorates capsaicinoid levels, because water deficit affects the phenylpropanoid pathway (Estrada et al., 1999).

Hydric stress can raise capsaicin levels by increasing activity of the enzymes phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H), and capsaicinoid synthetases (CS), all engaged in capsaicin biosynthesis (Sung et al., 2005). Monforte-González et al. (2010) found that application of nitrate can increase the capsaicin biosynthesis, but potassium application had not a significant effect on it. Ascorbic acid is very abundant in pepper fruits and is the main component of vitamin C. Its content is highly varying among cultivar and ripening stage (Bae et al., 2014).

Cytokinin is a hormone of plants that adjusts growth and developmental cycles. It has an important role on the chlorophyll status in plants (Lim et al., 2007). Exogenous treatment of N6-benzyl amino purine (BAP) on the eggplant increased its tolerance under salt stress by decreasing the O_2^- and malondialdehyde production rate (Wu et al., 2014). Application of BAP in the *in vitro* culture increased the comptothecin content in shoots and roots of *Ophiorrhiza rugosa* var. *decumbens* (Vineesh et al., 2007). It was reported that the application of BAP increased the shikonin formation in *Onosma paniculatum* cultured cell (Ding et al., 2004). So BAP has an exclusive effect on the plant growth and secondary metabolites production in the plants.

The results of the literature review suggest that abiotic and biotic parameters could affect the secondary metabolites production in plants. Up to now a research has not been done on the effect of cytokinin on the capsaicin production, so in this study, we investigated the effect of BAP on the capsaicin and ascorbic acid content in pepper.

MATERIALS AND METHODS

The experiment was performed in the Ataturk University under controlled greenhouse conditions. Seeds of *Capsicum annum* var. Ilica (pungent pepper) were germinated on the 86-celled styrofoam trays filled with peat, then the homogenous and healthy seedlings were transplanted to the pots in size 30 x 20 x 20 cm after thirty days. The pots were filled with a ratio of 2: 1: 1 (v: v: v) of soil: sand: manure having around 1.30 g cm^3 bulk density.

The greenhouse mean temperature and relative humidity were 24°C and 74%, respectively during the experiment. The cytokinin treatment included 0, 50 and 100 μM of N6-benzyl amino purine (BAP). The BAP solutions were prepared with distilled water containing 0.02% Tween 20 as a surfactant, and the control (without BAP) treatment just was contained distilled water and surfactant. Five days after seedling transplanting, the BAP solutions were sprayed to leaves in the afternoon and the foliar spraying continued every 10 days until the harvest. Half-strength Hoagland nutrient solution was used with irrigation (Rubio et al., 2011).

Marketable fruits were selectively harvested weekly from 55 days after transplanting until the end of the experiment. In addition, at the end of the experiment, one representative marketable pepper fruit (18 fruits) was selected from different plants and used for determining chemical analysis. Yield was determined by counting and weighing all fruits on each plant. Marketable fruit yield was determined according to the color, the health state, the shape, and the weight.

Capsaicin analysis

The samples were separated in a SHIM-PACK VP-ODS column (150 mm × 4.6 mm; Shimazu). For capsaicin, the eluent was a mixture of acetonitrile/1% acetic acid (2:1 v/v), and the flow rate was 1 ml/min. All eluates were monitored at 280 nm using a UV detector. External standards were prepared by dissolving commercial capsaicin (Sigma-Aldrich) in methanol and acetonitrile (Ogawa et al., 2015).

Ascorbic acid analysis

Five grams of well-homogenised sample were disrupted in a crucible mortar with quartz sand. To the macerate 50 mL of meta phosphoric acid (analytical grade) was gradually added and the mixture was then transferred to a 100 mL Erlenmeyer flask, closed with stopper and then filtered. The filtrate was purified in addition by passing through a 0.45 mm PTFE syringe filter before injection on HPLC column.

The analytical determination of ascorbic acid was performed on C18 Nautilus, 100-5, 150 × 4.6 mm column with gradient elution of 0.01 M KH_2PO_4 (A) and acetonitrile (B). The gradient elution started with 1% B in A and changed to 30% B in A in 15 min.; then, it turned to 1% A in B in 5 min. The flow rate was 0.7 mL/min. The highest absorption maximum of ascorbic acid under these conditions was detected at 265 nm. For quantitative determination of ascorbic acid, standard materials (Sigma-Aldrich, Budapest, Hungary) were used.

Stock solutions and then working solutions were prepared for each compound to make the calibration between concentration and peak area (Nagy et al., 2015).

Data analysis

A completely randomized experimental design was used in this study. Each treatment had 4 replications with 5 plants for each replication. Statistical analysis was carried out by SPSS version19 at $P \leq 0.001$, the mean separation was done following Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

As shown in the Figure 1, BAP treatments significantly increased the capsaicin content in fruits of pepper. Treatment of BAP (100 μM) had the highest effect on the capsaicin content, as compared to the untreated ones. Exogenous BAP treatment had a significant effect on the ascorbic acid content in the pepper fruits, by increasing the BAP concentration its content enhanced. Higher concentration of BAP (100 μM) increased the ascorbic acid content 22% in comparison to the untreated one (Figure 2). The cytokinin (BAP) application had not a positive effect on the fruit number in pepper plant. The BAP treatment (100 μM) decreased the fruit number but its lower concentration (50 μM) had not a significant effect as compared to the control plants (Figure 3).

Previous studies have shown that capsaicin biosynthesis exclusively is occurred in the fruit (Stewart et al., 2005; Stewart et al., 2007). Cytokinin has an important role in the adjustment of different biological processes, involving growth and development, furthermore, acclimation/adaptation to environmental conditions in plants. Li et al. (2016) reported that cytokinin application increased secondary metabolite production in *Morinda citrifolia*.

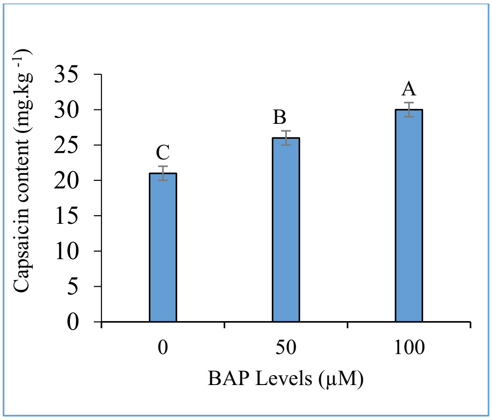

Figure 1. Effect of N6-benzyl amino purine (BAP) levels on capsaicin content in fruits of pepper. Data followed by a different letter were significantly different ($P \leq 0.001$) according to the Duncan Multiple Range Test

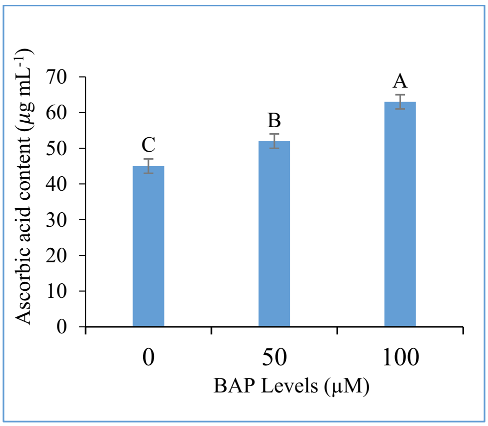

Figure 2. Effect of N6-benzyl amino purine (BAP) levels on ascorbic acid content in fruits of pepper. Data followed by a different letter were significantly different ($P \leq 0.001$) according to the Duncan Multiple Range Test

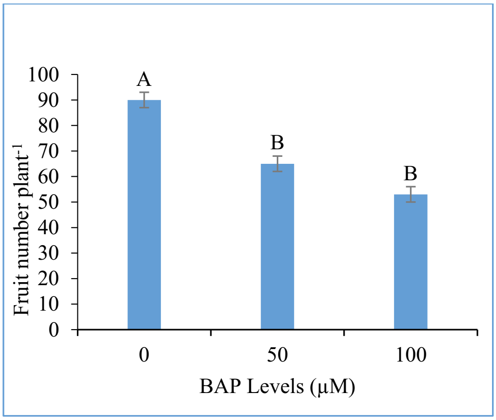

Figure 3. Effect of N6-benzyl amino purine (BAP) levels on number of fruit in pepper. Data followed by a different letter were significantly different ($P \leq 0.001$) according to the Duncan Multiple Range Test

Similarly, Govindaraju and Indra Arulselvi (2016) reported that BAP treatment enhanced the PAL and subsequently secondary metabolites production on *in vitro* media in *Coleus aromaticus*, without changes in plant genetic. In this study, exogenously BAP application increased the capsaicin content in fruits. It could be due to role of cytokinin on growth and development including meristem maintenance, vascular development, modulation of sink-source relationships and nutrient acquisition in roots.

BAP also play an important role in gene expression in capsaicin production. Moreover, Govindaraju and Indra Arulselvi (2016) reported that BAP application on *in vitro* propagation of *Coleus aromaticus* increased PAL gene expression, PAL directs phenylalanine (Phe) to secondary metabolism, deamination this amino acid to generate cinnamic acid, that could be converted to various phenolic compounds, involving capsaicin (Castro-Concha et al., 2016).

The ascorbic acid content with a gradual increase in fruit ripening was detected in hot pepper (Iqbal et al., 2013). Navarro et al. (2006) revealed that salinity decreased ascorbic acid content in pepper and methyl jasmonate application increased antioxidant capacity of strawberry fruits (Ayala-Zavala et al., 2005). In this study, BAP treatment increased ascorbic acid in Ilica pepper. It means that cytokinins have a role in ascorbic acid biosynthesis in pepper plants.

The previous study have been shown that cytokinin application had not a significant effect on the yield and dry matter accumulation in the ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Ghani et al., 2014). However, we found that BAP had a significant effect on the fruit number. In this regard, the cytokinin higher levels (50 and 100 μM of BAP) decreased the fruit number of pepper. The decline of fruit/plant could be due to its role in regeneration of shoot which it leads to the shadowing of plants on each other and it ultimately causes to decrease in the plants yield.

CONCLUSIONS

The literature review shows that capsaicin is responsible for pungent sensation in pepper cultivators and the fruit placenta is a main place for capsaicin production. BAP treatment had a significantly effect on capsaicin (CAP) production and increased CAP and ascorbic acid content in pepper.

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