Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2012, 4(1):82-85

Notulae Scientia Biologicae

Hinokitiol Enhanced Vegetative Growth Parameters of Tomato cv. 'Falkato' Compared with Salicylic Acid and Paclobutrazol under *In Vitro* Salinity Condition

Behrooz ESMAEILPOUR, Hassan MALEKI LAJAYER*, Leila KESHVARZI, Younes POURBEYRAMI HIR

*University of Mohaghegh Ardabili, Faculty of Agriculture, Department of Horticultural Science, Ardabil, Iran; malekihassan@yahoo.com (*corresponding author)*

Abstract

The aim of this study was to assess the potential *in vitro* effect of hinokitiol on improvement of tomato seedling resistance to salinity stress. Effect of hinokitiol was compared with two anti-stress compounds, salicylic acid and paclobutrazol. Leaf numbers, shoot and root fresh weight and root fresh weight were recorded after about 8 weeks. Salt stress was accomplished by application of two levels of pure NaCl (50 and 100 mM) on MS basal medium. The treatments consisted of different concentrations of hinokitiol (0, 1, 5 and 10 ppm), paclobutrazol (0, 1, 2 and 4 μM) and salicylic acid (0, 0.01, 0.1 and 1 mM). Results revealed that salinity blocked seed germination in media containing only 100 mM of pure NaCl without any treatment. In general all three compounds increased tomato seedling growth, indicating these compounds are able to alleviate the negative effect of salinity on tomato plants. However, Hinokitiol was the most efficient compound. Compared with SA, application of hinokitiol significantly increased leaf numbers, shoot length and shoot and root dry weight. Also, media containing different concentrations of hinokitiol produced higher root and shoot fresh weight than control and other treatments. Future physiological studies are needed to clarify the mechanism of induction of salt tolerance activity by hinokitiol.

Keywords: fresh weight, salinity, vegetative growth parameters

Abbreviations: HIN (Hinokitiol), SA (Salicylic acid), PBZ (Paclobutrazol)

Introduction

One of the most important factors that dictate the distribution of many plant species is their ability to withstand environmental stress including variation in salinity and available moisture. Plants generally respond the environmental stress by activating defense mechanisms and adjusting their cellular metabolism (Mehdy, 1994).

Stresses may be of a biotic (infection caused by fungi, bacteria and viruses) or abiotic (water, temperature or ionic stresses) nature. Plants initially perceive environmental stresses and activate a range of defense mechanisms (Sticher *et al.*, 1997). Abiotic stress conditions favor the accumulation of reactive oxygen peroxide , such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (H_2O_2). The alleviation of oxidative damage and increased resistance to environmental stresses is often correlated with an efficient anti oxidative system which may be induced or enhanced by application of chemical such as salicylic acid or paclobutrazol. Recently considerable interest has been aroused by the ability of PBZ and SA to produce a protective effect on plants exposed to various abiotic stress factors. SA is considered to be a hormone-like substance that is important in regulation of plant growth and development processes such as ion uptake and transport, photosynthesis rate and transpiration (Khodhary, 2004). This compound in some cases serve as a plant hormones, regulate the synthesis of anti oxidant enzymes during abiotic and biotic stress (Senaratna *et al.*, 2003). The application of SA has resulted in tolerance of plants to many biotic and abiotic stresses including fungi, bacteria, viruses (Delany *et al.*, 1994), chilling (Janda *et al.*, 1999), drought and extreme temperatures (Senaratna *et al.*, 2003).

The physiological effect of paclobutrazol in plant which undergo abiotic stress has been reported. This compound is currently registered for use on food crops such as apples, peaches and citruses but it is very effective in controlling the growth of many other crops and may offer an alternative for use in vegetable production (Fletcher *et al.*, 2000). PBZ increased resistance against environmental stresses such as drought, salinity and high temperature (Berova and Zaltav, 2000). These beneficial effects could be attributed to a decrease in endogenous gibbrelin level and transient increase in abscisic acid level and enhancement of anti oxidant enzymes activity (Tekalign and Hammes, 2005).

Hinokitiol (β-tujaplicin) is a tropolon-related compound purified from the wood of *Chamaecyparis obtusa*, *Thuja plicata*. Hinokitiol has been used as antibacterial agent in foods and cosmetics due to low toxicity in ani-

Received 05 November 2011; accepted 22 January 2012

mals (Arimal *et al*., 2003). The mechanism of antimicrobial and insecticidal activity of tropolone and hinokitiol is unknown but it was documented that tropolone greatly inhibited polyphenol oxidase and tropolone and hinokitiol showed inhibitory activity toward metalloproteases such as carboxypeptidase A and collagenase (Morita *et al*., 2004, Saniveska and Saniewski, 2007).

Inhibitory effects of hinokitiol on some plant growth such as seed germination have been reported (Sakagami *et al*., 2000). Moreover, hinokitiol can block the biosynthetic pathway of enzymes such as ACC-synthase and ACC-oxidase (Zobayd *et al*., 2001).

Material and methods

Plant material and media preparation

Tomato seeds cv. 'Falkato' were used as explants cultured in tissue culture laboratory of Mohaghegh Ardabili. MS basal medium supplemented with 3% sucrose, 0.8% agar was used as cultural medium. Two concentrations of pure NaCl (50 or 100 mM) were added to cultural media to provide a saline condition. Treatments consisted of different concentrations of hinokitiol (0, 1, 5 and 10 ppm), paclobutrazol (0, 1, 2 and 4 μM) and SA (0, 0.01, 0.1 and 1 mM) and all treatments were added to cultural medium before autoclaving at 121°C. Hinokitiol was dissolved in 70% ethanol. Cultures were kept at 25°C temperature and 16/8 day/night photoperiod in growth chamber.

Explants and media sterilization

To obtain sterile culture, seeds were leached with running water for 15 minutes and disinfested by 70% ethanol and 3% sodium hypochlorite for 45 second and 20 min, respectively; then seeds was leached three-time with distilled water. Cultural media sterilized by autoclaving at 121°C.

Data recording and analysis

After two month seedling growth indices including leaf numbers, shoot and root fresh weight and shoot length were recorded. This experiment was carried out based on completely randomized design with 5 replicates. Data were subjected to analysis of variance by SAS 9.12 software and means were separated by Duncan multiple range test at P≥0.05.

Results

Application of NaCl (especially at100 mM) to MS basal medium adversely influenced tomato plant growth pattern by inhibition from seeding growth. Also, results of this experiment revealed that hinokitiol, PBZ and SA significantly increased tomato seedling tolerance against salinity stress under *in vitro* culture, (Tab. 1 and 2).

Leaf numbers

Fig. 1 shows the effects of treatments on leaf numbers on media containing both salinity levels (50 and 100 mM). It can be clearly seen that all treatment significantly increased tomato seedling growth except 1 mM of SA, inhibited from seedling growth on both salinity levels. Hinokitiol increased leaf numbers, this compound at rate of 10 mM accounted for the most leaf under high salinity level, while on media containing lower level of NaCl, seedling treated by low concentration of PBZ produced more leaf than other treatments. SA at high concentration

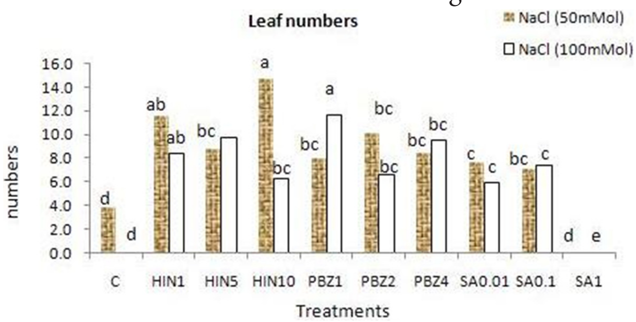

Fig. 1. Effect of hinokitiol, salicylic scid and paclobutrazol on leaf number

Note: Numbers beside the abbreviations indicating the tested concentrations of compounds

Tab. 1. The analysis of variance of salt levels and treatments on growth parameters of tomato on media containing of 100 mM of NaCl

Source	Df	Mean Square			
		Leaf numbers	Shoot length (cm)	Shoot F.W (mg)	Root F.W (mg)
Model	9	6.67^{**}	3.36^{**}	210^{**}	22^{**}
Error	40	0.2	0.07	4.95	0.50
CV		16	12	19.2	17.3

Tab. 2. The analysis of variance of salt levels and treatments on growth parameters of tomato on media containing 50 mM of NaCl

Source	Df	Mean Square			
		Leaf numbers	Shoot length (cm)	Shoot F.W (mg)	Root F.W (mg)
Model	9	4.95^{**}	2.58^{**}	165.7^{**}	24.9^{**}
Error	40	0.19	0.10	9.17	2.35
CV		14	13	21.3	27

Note: ** and NS indicate significant or nonsignificant difference at *P*≤%1

(10 mM) showed inhibitory effect on tomato seedling growth.

Shoot length

Shoot length of seedlings reduced by application of hinokitiol, SA and PBZ on media supplemented with 50 mM of NaCl, in contrast, under high salinity level hinokitiol alleviated the inhibitory effect of salinity and increased shoot length of seedling. Increasing the concentration of hinokitiol up to 5 ppm increased shoot length but higher level (10 ppm) of this compound reduced shoot length. Different concentrations of PBZ produced seedlings with same shoot length, while higher concentrations of SA produced seedlings with shorter shoot than that of lower concentrations (Fig. 2).

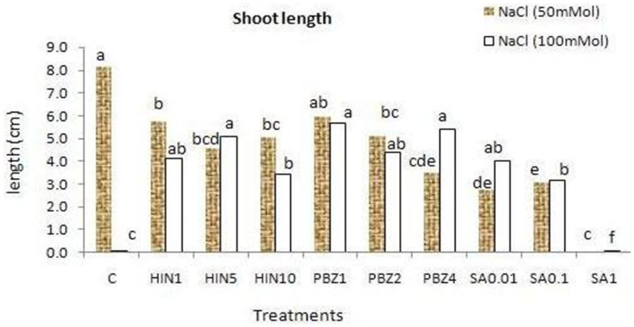

Fig. 2. Effect of hinokitiol, salicylic scid and paclobutrazol on shoot length

Shoot fresh weight

It can be clearly seen that hinokitiol on media containing of 50 mM of NaCl, produced seedling with higher shoot fresh weight than other treatments. However, higher concentration of this compound declined shoot fresh weight. SA at higher concentration inhibited from seedling growth and subsequently decreased shoot fresh weight. Seedling produced on media with high salinity level showed different results. An increase in hinokitiol concentration decreased shoot fresh weight, especially at 10 ppm. There were conflicting results about media supplemented with PBZ, both higher and lower concentration of this compound produced shoots with high fresh weight of those of moderate concentration (Fig. 3).

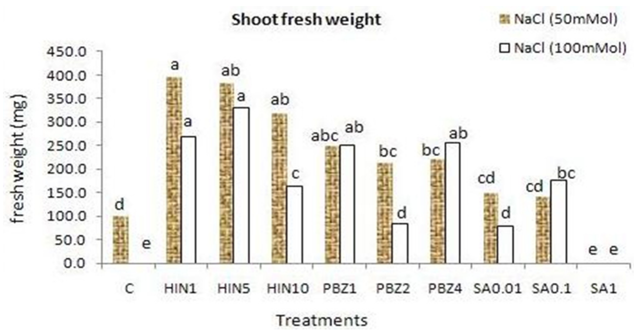

Fig. 3. Effect of hinokitiol, salicylic scid and paclobutrazol on shoot fresh weight

Root fresh weight

As shown in Fig. 4, hinokitiol under both salinity levels significantly ($P \geq 0.5$) increased root growth and seedling grown on media containing of 10 and 5 ppm hinokitiol had the highest fresh weight under low and high salinity levels, respectively. Other treatments had not any positive effect on root growth under lower salinity level. Whereas, PBZ and SA increased root fresh weight under high salinity level.

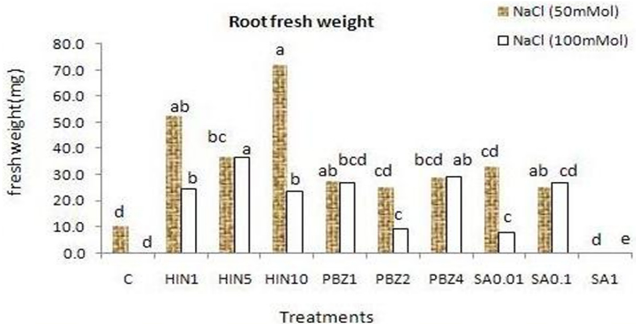

Fig. 4. Effect of hinokitiol, salicylic scid and paclobutrazol on root fresh weight

Discussion

Media supplemented with hinokitiol, SA and PBZ positively affected tomato plants growth pattern under both salinity levels. Two salinity levels affected plant growth, similarly. This is of primary importance from a point of view since hinokitiol, SA and PBZ could increase plants tolerance against to either low or high salinity condition. Previously the positive effects of PBZ and SA on plants growth under salinity stress have been reported (Tekalign and Hammes, 2005). According to Borsani *et al.* (2001) SA multiplies the ROS generation under stress and a direct physiological effect of SA is the alternation of antioxidant enzyme activities. Exogenous SA could regulate the activity of antioxidant enzymes and increase plants tolerance to abiotic stress (He *et al.*, 2002). Also, Marshal *et al.* (2000) reported that PBZ anti-stress activities could be attributed to its antioxidant activity.

The highest shoot and root fresh weight were recorded on media supplemented with different concentrations of hinokitiol. The most effective rate of this compound was 5 ppm. The pronounce effects of tropolene and hinokitiol-related compounds in biological systems including strong inhibition of plant growth, antimicrobial and insecticidal activity. The mechanism of the antimicrobial and insecticidal activity of tropolone and hinokitiol is unknown but it was well documented that tropolone greatly inhibited polyphenol oxidase (Morita *et al.*, 2004). Inhibitory effects of hinokitiol on enzymes have been reported (Rabbany and Mizutani, 1998). For instance hinokitiol inhibited ethylene production through suppression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and oxidase (Rabbany and Mizotany, 1998). Tropolone and hinokitiol greatly inhibited the formation of red pigment in wounded scales of *Hippeastrum*; probably as strong inhibitors of polyphenol oxidase block the oxidation of colorless flavans to oxidized red-coloured flavans after wounding. The role of oxidative stress in the production of hinokitiol by *Cupressus lusitanica* suspension culture is also presented (Saniveski *et al*., 2007). Moreover, in present study it was shown that exogenous hinokitiol decreased the negative effects of salinity in tomato plants. This evidence may be serve as signs of interference of this compound in alleviating of stresses but more investigation is needed for confirming of these observations.

Acknowledgments

The research was supported by Horticultural Science Department of Agriculture Faculty of Mohaghegh Ardabili University.

References

Arimal Y, Nakai Y, Hayakawa R, Takrshi N (2003). Antibacterial effect of β-thujaplicin on staphylococci isolated fromatopic dermatitis: relationship between changes in the number of viable bacterial cells and clinical improvement in an eczematous lesion of atopic dermatitis. J Antimic Chem 51:113-122.

Berova M, Zlatev Z (2000). Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regul 30:117-123.

Borsani O, Valpuesta V, Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol 126:1024-1030.

Delany TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J (1994). A central role of salicylic acid in plantdisease resistance. Science 266:1247-1250.

Flecher RA, Sankhla GN, Davis T (2000). Triazoles as plant growth regulators and stress protectants. Hortic Reviews 24:55-122.

He CY, Zhang JS, Chen SY (2002). A soybean gene encoding a proline-rich protein is regulated by salicylic acid, an endogenous circadian rhythm and by various stresses. Theor Appl Gen 104:1125-1131.

Janda T, Szalai G, Tari I, Paldi E (1999). Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. Planta 208:175-180.

Khodary SEA (2004). effect of SA on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Agricult Biol 1:5-8.

Maleki LH, Esmaielpour B, Chamani E (2011). Hinokitiol and Activated charcoal influenced microtuberization and growth of potato (*Solanum tuberasum* cv. 'Agria') plantlets *in vitro*. AJCS (in press).

Marshall JG, Rutledge RG, Blumwald E, Dumbroff EB (2000). Reduction in turgid water volume in jack pine, white spruce and black spruce in response to drought and paclobutrazol. Tree Physiol 20:701-707.

Mehdy MC (1994). Active oxygen species in plant defense against pathogens. Plant Physyol 105:467-472.

Morita Y, Matsumura E, Okabe T, Fukui T, Ohe T Ishida N, Inamori Y (2004). Biological activity of beta-dolabrin, gamma-thujaplicin, and 4-acetyltropolone, hinokitiol-related compounds. Biol Pharm Bull 27:1666-1669.

Rabbany ABMG, Mizutani F (1998). Effect of tropolon and hinokitiol on *in vitro* activities of ACC syntase and oxidase in wounded winter squash mesocarps. J Japan Soc Hort Sci 67:213-215.

Sakagami Y, Inamori Y, Isoyama N, Tsujibo H, Okabe T, Morita Y, Ishida N (2000). Phytogrowth-Inhibitory activities of β-dolabrin and γ-thujaplicin, hinokitiol-related compounds and constituents of *Thujopsis dolabrata*. Biolo Pharm Bul 23 (5):645-648

Saniewska A, Saniewski M (2007). The inhibitory effect of tropolon and hinokitiol on the mycelium growth of *Phoma narcissi in vitro*. Acta Arobot 60 (1):107-112.

Senaratna T, Merrit D, Dixon K, Bunn E, Touchell D, Sivasithamparam K (2003). Benzoic acid may act as thefunctional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. Plant Growth Regul 39:77-81.

Sticher L, Mauch-mani B, Metraux JP (1997). Systemicacquired resistance. Annu Rev Phytopathol 35:235-270.

Tekalign T, Hammes PS (2005). Response of potato grown in a hot tropical low land to applied paclobotrazol. II. Tuber attribute. New Zealand J Crop Hort Sci 33: 43-51.

Zobayd SM, Armstrong A, Armstrong WJ (2001). *In micro* propagation of potato: Evaluation of closed, diffusive and forced ventilation growth and tuberization. J Zhejiang Univer 32(1):36-40.