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The Effect of Putrescine on Date Bunch Fading Disorder

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ABSTRACT: Polyamines are nitrogen-rich compounds and induce resistance in plants against pests, diseases and physiological disorders. This study was performed to investigate the effect of putrescine spray solution (a compound derived from free polyamines group) to reduce the amount of damaged fruits caused by date bunch fading disorder in Mazafati date palms in Kerman province. In this study the percentage of damaged fruits caused by date bunch fading disorder, length, diameter and weight of fruit, fruit pulp to seed ratio, total soluble solids of fruit, percentage of fruit total sugar, pH, titratable acidity, percentage of fruit protein, percentage of fruit moisture and yield were evaluated. The obtained results showed application of 12mM putrescine concentrations was effective on reduction of damaged fruits caused by date bunch fading disorder. Furthermore, application of 12mM putrescine concentration caused increase on diameter of fruit, weight of fruit and yield in experimental trees. All putrescine treatments caused increase in fruit pulp to seed ratio. Application of 4mM putrescine caused significant increase on length of fruit and content of fruit moisture. Putrescine application caused reduction in total soluble solids, total sugar and percentage of fruit protein. Putrescine application showed no effect on titratable acidity and pH.

Key words: Bam, date bunch fading disorder, fruit, putrescine

INTRODUCTION

Date fruit is one of Iranian valuable horticultural products on which the income and occupation of the majority of the stares' southern provinces' residences are depended. This product plays an important role in regard with food security, national economics, entrepreneurship, environmental conservation and sustainability, export and exchange technology in the state. In the recent years, the date bunch fading disorder has been the most important challenge of date production in Iran. This disorder first was reported in 1989 from Kahnooj area in Kerman Province. However, none of the main date-producing countries of the world have reported this disorder yet. In Kerman province, Mazafati date cultivar is very susceptible to this disorder (Panahi *et al*., 2011; Panahi *et al*., 2012).

The date bunch fading disorder may be observed in the fruits in their Kharak-to-Rutab transformation stage. The symptoms of this disorder first appear in form of fruits wilt. Simultaneously with fruits wilting, tip of bunches' axis becomes shriveled, then brown-color spots appear on them and gradually whole axis of bunch and fruits turn shriveled. In some cultivars (Mazafati) a brown-color strip appears on the tail of the main bunch which finally becomes dried and woody (Panahi *et al*., 2011; Panahi *et al*., 2012).

There are several hypotheses about causes of outbreak and development of this disorder, the most important of which include climatic causes, pathogens- especially fungal agents, nutrient deficiencies or disorders and lack of observing optimal cultivation and management principles in the groves (Pezhman, 2004). Mohammadi & Moghtaderi (2005) reported that generally reduced relative humidity and increased temperature are among the main causes of date bunch fading disorder outbreak. Panahi (1999) considers the causes of the disorder outbreak to be related to the time during which the plant is subject to environmental sever and sudden stresses.

Polyamines exist in the plant in free form or combined or connected to other compounds, that, in the case of being connected to other compounds, they may be soluble or insoluble in water. Soluble joined polyamines are present in plants widely, and are produced in the result of combination with phenols (Rastogi & Davie, 1991). Putrescine, spermine and spermidine polyamines are organic polycations with low molecular weight and aliphatic groups, which possess different hydrocarbon rings and include two or more amino groups (positives charges' agent), that are widely present in all living organisms. Polyamines and their biosynthesising enzymes play roles in a wide area of metabolic processes in plants including cell division, organogenesis, growth and protection against different kinds of environmental stresses (Kaur-Sawhney *et al*., 1982) Polyamines play an important role in plants' defensive reaction against different stresses (Prabhavathi & Rajam, 2007). It has been reported that increased rate of putrescine results in reduced cellular potassium (Shen *et al*., 2000).

Salinity and osmotic stresses cause increase in polyamines due to the stress imposed on the plant. Deficiency of Potassium and also Calcium and Magnesium results in increased putrescine. Generally, polyamines are effective in protecting the membrane against oxidative stresses by collecting free radicals (Mirdehghan *et al*., 2007). Khassem *et al*. (2012) observed that foliar application of putrescine by the concentration of 8mM on Barhi date cultivar caused delay in fracture of the fruit's green colour, significantly. It is believed that polyamines have anti-aging property (Kaur-Sawhney *et al*., 1982) Considering the role, polyamines play in protection against different environmental stresses, their role in the processes related to growth (Shiozaki *et al*., 2000), fruit formation improvement and fruit size and quality (Ziosi *et al*., 2003; Paksasorn *et al*., 1995), and their anti-ethylene role (Galeston & Sawhney, 1990; Valero *et al*., 2002), and also taking into consideration the results of the previous researches which believe the severe and sudden stresses to be effective on the disorder's outbreak (Panahi, 1999) making an allowance for the confirmation of the role of relative humidity and temperature variations in the disorder's exacerbation (Darini *et al*., 2001; Mohammadi & Moghtaderi, 2005), and with regard to this that external application of putrescine before fruit harvest in form of foliar application is an appropriate method for increasing the internal polyamines. According to these hypotheses, studying the application of putrescine towards reducing the damage percentage of bunch of Mazafati date cultivar in Bam area seemed to be essential.

MATERIAL AND METHODS

This experiment was carried out in a randomized complete block design with four treatment levels (0, 4, 8 and 12mM) of putrescine and three replicates for two years (2010 and 2011) in Narmashir near Bam area located in Kerman province, Iran. Foliar application was performed at the beginning of fruits' color change (at the beginning of Kharak stage).

Measurements of studied characteristics

The measurements of studied characteristics included the disorder damage percentage, length, diameter and weight of the fruit, pulp to seed ratio, total sugar percentage, TSS, pH, titratable acidity, protein, moisture and yield.

After note takings, the gathered data were analyzed using MSTATC software, and means comparison test was performed through Duncan's multiple range tests.

Physical analysis

At the harvesting time, 20 full Rutab fruits per bunch were randomly collected for Physical measurements. Fruit weight, fruit diameter, fruit length, fruit pulp weight, and seed weight were recorded independently in each of the 20 fruits per replicate. A homogenous sample was prepared from each 20 fruits replicate for measurements of chemical characteristics.

Date bunch fading disorder (%)

In order to calculate date bunch fading disorder, 50 fruits were picked randomly from four bunches in four direction of each palm.

pH

The pH of palm sugar concentrate was measured by a pH meter. Calibration was standardized using pH 7.0 and 4.0 buffers (A.O.A.C, 2000). Each sample was measured in three replications.

Total acidity

Total acidity was determined by titration method with 0.1N sodium hydroxide, which was standardized using potassium hydrogen phthalate (3.2% w/v) prior each titration. A few drops of 1% phenolphthalein were used as an indicator. A sample of palm sugar concentrate (15ml) was titrated with 0.1N sodium hydroxide until reached end point and persisted for 15-20 seconds, and the color was changed to pink color at pH 8.1. Three titrations were performed for each sample. The percentage of total acidity obtained as
equivalent to lactic acid content (A.O.A.C, 2000).

Total soluble solids

Total soluble solids of palm sugar concentrate were measured using Atago hand-held refract meter. All samples were measured in three replications and reported in degree brix (Brix^{o}) (A.O.A.C, 2000).

Total sugar content

The determination of total sugars by Lane and Eynon volumetric method was employed. This method consists of the chemical preparation, sample preparation and titration method (A.O.A.C, 2000).

Protein content

Content of nitrogen (N) was measured by the Kjeldahl method (AOAC, 1990). The crude protein (CP) was calculated as N × 6.25.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results of combined variance analysis, and average of the characteristics under study during two experimental years of 2012 and 2013 in regard with the treatments' effects have been presented in tables 3 and 4.As it was observed from the results, the trees treated with foliar application of putrescine showed a lower severity of date bunch fading disorder in compare to the control. Among different levels of putrescine's concentrations, the concentration of 12mM showed a better result in reducing the damage percentage of date bunch in compare to other treatments. This concentration, by the average of 39.67 percent, showed about 19 percent reduction in disorder's severity in compare to the control.

Reduced damage percentage of the bunch in the result of putrescine application can be attributed to the role of polyamines play in protecting the plant against different environmental stresses (Kaur-Sawhney *et al*., 1982; Prabhavathi & Rajam, 2007; Serrano *et al*.,2004; Tiburcio *et al*.,1997) such as high temperature (Liu *et al*., 2006) Polyamines also play role in protecting plants against lipids' peroxidation as passive defensive mechanism of cell in resisting against oxidative damage (Roy *et al*., 2005) This group of compounds have antioxidant properties, ability to neutralize acid, and ability to stabilize the cell wall and membrane (Hussein *et al*., 2006), and to prevent its destruction under stress condition (Liu *et al*., 2007) Some researchers attribute the disorder's outbreak to the time when the plant is under severe and sudden environmental stress (Panahi, 1999). This research work tried to increase the internal polyamines by foliar application of putrescine before fruit harvest (Mirdehghan *et al*., 2007), in order to make the plant resistant against stresses such as reduced relative humidity and increased temperature (Panahi *et al*., 2011) which contribute to the disorder exacerbation. Putrescine application significantly increased the fruit length of Mazafati date cultivar (Table 1). Among different concentrations of putrescine, the concentrations of 4mM and 12mM showed to have the highest effect on increasing the fruit length. Application of putrescine by the concentration of 4mM is more justified economically. In a research reported by Ziosi *et al*. in 2003, by analysing and studying the activity of biosynthesising enzymes of polyamines in mesocarp tissue during the growth steps of peach fruit, they found that polyamines play an important role in fruits growth and ripening, and those polyamines are present in high levels during the cell division step. Accordingly, considering the polyamines' role in regulating different growth processes of fruits, supplying polyamines externally can contribute to improvement of fruit formation, and fruit size and quality, and also have positive effect on the after-harvest disorders.

Putrescine application caused increase in fruit diameter, fruit weight, pulp to seed ratio and yield of Mazafati date cultivar (Table 1). Among different concentrations of putrescine, the concentration of 12mM showed to have the highest effect on fruit diameter, fruit weight and crop yield. In studying the different concentrations, it was found that all treatments had identical effect on pulp to seed ratio. The results of this research work indicated that, generally, putrescine affects fruit length, fruit diameter, fruit weight and crop yield, significantly.

Table 1. Analysis of variance of the effect of the treatments on the measured traits during 2012 – 2013.

Mean Squares (MS)						df	Source of variations
Date bunch fading disorder	**Yield**	**Pulp to seed ratio**	**Weight of fruit**	**Diameter of fruit**	**Length of fruit**		
**	**	**	**	**	n.s		Year (A)
61.042	10.542	8.365	1014.25	1.99	20.175		Error
**	**	**	**	*	**		Treatment (B)
n.s	n.s	n.s	**	n.s	n.s		(A) ×(B)
11.486	20.486	3.108	724.294	6.73	8.191		Error
7.56	24.52	15.84	19.32	8.07	5.37		CV (%)

n.s., non-significant; *, significant at P≤0.05; **, significant at P≤0.01.

Similar results regarding putrescine's effect on fruit's physical properties have been reported by other researches such as Khassem *et al*. (2012) and Stern *et al*. (2006) who all found that polyamines application results in fruit's increased size and improved physical properties, due to remarkable development of cells and increased absorption potential of carbohydrates in fruits (Khassem *et al*., 2012). Valero *et al*. (2002) reported that polyamines are essential for growth, cell differentiation, and intracellular concentration increase during cell proliferation period. During their research, Paksasorn *et al*. (1995) found that foliar application of polyamines on apple trees contributes to increased fruit's growth. Therefore, polyamines act as a source of nitrogen for stimulating the growth. In another research, by analysing and studying the activity of the biosynthesising enzymes of polyamines (Arginine decarboxylase, Ornithine decarboxylase, and S-adenosylmethionine decarboxylase) in the mesocarp tissue during the growth steps of peach fruit, it was found that polyamines play an important role in fruits' growth and ripening, and that polyamines' levels are high during the cell division step. Accordingly, considering the polyamines' role in regulating different growth processes of fruits, supplying polyamines externally can contribute in improvement of fruit formation, fruit size and crop yield (Ziosi *et al*., 2003). In another research, use of polyamines by foliar application method on apricot fruit resulted in increased yield and fruit size (Ali *et al*., 2010). In addition, polyamines are able to cooperate with non-ionic molecules such as DNA and RNA and proteins which play role in many processes inside the plant including cell division and enlargement and fruit growth and ripening. Ali *et al*. (Ali *et al*., 2010) reported that foliar application of polyamines on apricot fruit causes increased yield and fruit size.

Putrescine's different levels resulted in reduction of total sugar content and total soluble solids of the fruit (Table 2), in a way that the fruits in control treatment had higher levels of total sugar and total soluble solids in compare to the fruits in other treatments. Results of the studies carried out by Khassem *et al*. (2012) also showed that putrescine application caused reduced levels of total soluble solids and total sugar of Barhi date cultivar. Putrescine application before harvest on mango fruit (Malik *et al*, 2005) resulted in reduced TSS level of the fruit, but on apple fruit (Costa & Bangni, 1983) and litchi fruit (Mitra & Sanyal, 1990), were resulted in increased TSS level.

In the present research, putrescine application showed no significant effect of fruit's pH (Table 2). In studying the effect of exogenous putrescine on the after-harvest life time of 'Salva' strawberry cultivar, it was found that putrescine had no significant effect on the fruits' pH and titratable acidity (Zokaee Khosroshahi & Esna-Ashari, 2008).

Table 2. Analysis of variance of the effect of the treatments on the measured traits during 2012 – 2013.

Mean Squares (MS)					df	Source of variations
Acidity	**Protein**	**pH**	**TSS**	**Total sugar**		
n.s	**	0.107	**	n.s	1	Year (A)
0.0001	0.307	0.027	11.54	18.525	4	Error
n.s	**	0.003	**	*	3	Treatment (B)
n.s	n.s	0.046	n.s	n.s	3	(A) ×(B)
0.0001	0.048	0.009	20.73	41.12	12	Error
10.28	16.93	1.33	8.5	9.18		CV (%)

n.s, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3: Biennium mean comparison of different levels of putrescine treatments on the measured traits (2012-2013).

Putrescine (Mm)	Length of fruit (Cm)	Diameter of fruit (Cm)	Weight of fruit(g)	Pulp to seed ratio	Yield (Kg)	Date bunch fading disorder (%)
Control	50.42^{b}	30.05^{b}	118^{b}	9.067^{b}	11.17^{c}	57.83^{c}
4	54.67^{a}	31.85^{ab}	139.8^{ab}	11.5^{a}	$16b^{c}$	43.33^{b}
8	53.5^{ab}	33.00^{a}	148.8^{ab}	11.47^{a}	18.67^{b}	41.5^{b}
12	54.58^{a}	33.68^{a}	158.3^{a}	12.41^{a}	28^{a}	39.67^{a}

Values in each column (Mean) with the same alphabet are not significantly different at 5% level whereas values with different alphabets are significant at 5% level.

Table 4: Biennium mean comparison of different levels of putrescine treatments on the measured traits (2012-2013).

Putrescine (Mm)	Total sugar (%)	TSS	pH	Protein (%)	Acidity (%)
Control	75.54^{a}	59.03	7.05^{a}	1.587^{a}	0.085^{a}
4	70.53^{ab}	55.6^{ab}	7.033^{a}	1.347^{ab}	0.086^{a}
8	69.42^{ab}	52.55^{b}	7^{a}	1.022^{c}	0.080^{a}
12	63.96^{b}	45.82^{c}	7.017^{a}	1.203^{bc}	0.088^{a}

Values in each column (Mean) with the same alphabet are not significantly different at 5% level whereas values with different alphabets are significant at 5% level.

Putrescine application significantly reduced protein content of Mazafati date cultivar (Table 2). In studying the effects of mutual application of spermidine and salinity stress on pepper plant, Noohpisheh and Kalantari (2012) observed that spraying the leaves by spermidine solution by the concentration of 2mM significantly increased the leaves' protein content in compare to the control.

In studying the effect of putrescine application on the moisture content of the fruit of Mazafati date cultivar, it was found that the fruits treated by 4mM concentration of putrescine significantly had higher moisture content in compare to the control. According to the results of the study conducted by Zokaee Khosrowshahi & Asnaashari (2008), putrescine reduced ethylene production and fruit's rate of water loss, and prevented their tissues' softening during storing. Titratable acidity consists of total acidity of fruit juice which is measured based on the fruit's dominant organic acid. In date fruit, the dominant organic acid is Malic acid. Results of studying the effects of putrescine application on titratable acidity of the fruit of Mazafati date cultivar showed that this compound have no significant effect on date's acidity as all treatments were place at a same level in this regard. Results of studying carried out by Khassem *et al*. (2012) showed that putrescine application caused increased acidity in the fruits of Barhi date cultivar.

CONCLUSIONS

According to the abovementioned findings, foliar application of putrescine on the fruits at the beginning of Kharak stage in Mazafati date cultivar trees at Bam region caused reduction of damage percentage of date bunch fading disorder (by 19%), and also contributed to increase of trees' yield (by 16kg). As it was discussed, different concentrations of putrescine increased fruit length, fruit diameter, fruit weight, fruit moisture content and pulp to seed ratio, and reduced fruit's protein content, total sugar and total soluble solids in compare to the control. Titratable acidity and pH of the fruit were not affected by putrescine treatment.

REFERENCES

Ali EA.M, Sarrwy SMA, Hassan HSA. (2010). Improving Canino Apricot Trees Productivity by Foliar Spraying with Polyamines. *Journal of Applied Sciences Research* **6**(9): 1359-1365.

A.O.A.C. (2000). Official Methods of Analysis. Association of Official Analytical Chemists. 17th ed. Gaithersburg, Maryland, U.S.A.

Costa G, Bangni N. (1983). Effects of polyamines on fruit set of apple. *HortScience* **18**(1), 50-61.

Darini A. (2001). Evaluation the role of climatic factors on drying blossom of date palm. Final Report of Jiroft Agriculture Research Centre, Jiroft, Iran. P. 20.

Galeston AW, Sawhney RK. 1990. Polyamines in plant physiology. *Plant Physiology* **94**, 406-410.

Hussein MM, Nadia E, EL-Gereadly HM, EL-Desuki M. (2006). Role of putrescine in resistance to salinity of pea plants (*Pisum sativum* L.). *Applied Science Research* **2**, 598-604.

Kassem HA, Al-Obeed RS, Ahmed MA. (2012). Effect of bioregulators preharvest application on date palm fruit productivity, ripening and quality. *African Journal of Agricultural Research* **7**(49), 6565-6572.

Kaur-Sawhney H, Shih L, Flores HE, Galston AR. 1982. Relation of polyamine synthesis and titer to aging and senescence in oat leaves. *Plant Physiology* **69**(2), 405-410.

Liu J, Kitashiba H, Wang J, Ban Y, Moriguchi T. (2007). Polyamines and their ability to provide environmental stress tolerance to plants. *Plant Biotechnology* **24**, 117-126.

Liu J, Nada K, Pang X, Honda C, Kitashiba H, Moriguchi T. (2006). Role of polyamines in peach fruit development and storage. *Tree Physiology* **26**,791-798.

Malik AU, Singh Z, Khan AS. (2005). Role of polyamines in fruit development, ripening, chilling injury, storage and quality of mango and other fruits: a review. Proceedings of International conference on Mango and Date Palm: Culture and Export, Malik *et al*., (Eds.), 20th to 23th June 2005, University of Agriculture, Faisalabad, P. 182-187.

Mirdehghan SH, Rahemi M, Castillo S, Martínez-Romero D, Serrano M, Valero D. (2007). Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology* **44**(1), 26-33.

Mitra SK, Sanyal, D. 1990. Effect of putrescine on fruit set and fruit quality of Litchi. *Gartenbauwissenschft* , **55**, 83-84.

Mohammadi H, Moghtaderi G. (2005). Relationship between climatic parameters and drying blossom of date palm disorder. *Desert* **2**, 339-348.

Noohpisheh Z, Kalantari KM. (2012). The interaction effects of spermidine application and salinity stress in pepper plants. *Journal of Iran's Biology* **24**(6), 848-857.

Paksasorn A, Hayasaka T, Matsui H, Ohara H, Hirata N. (1995). Relationship of polyamine content to ACC content and ethylene evolution in Japanese apricot fruit. *Journal of Japan Society of Horticultural Science* **63**(4), 761-766.

Panahi B, Damankeshan B, Asaadi M. (2012). Chemical effects of putrescine on date palm fruits. Proceedings of First National Conference on Dates and Food Security, Ahvaz, Iran. P. 4.

Panahi B, Damankeshan B, Asaadi M. (2012). Technical recommendations to reduce the damage of drying and wilting blossom of date palm disorder. Proceedings of scientific festival on dates, Bam, Iran. P. 256-265.

Panahi B, Damankeshan B, Asaadi M. (2011). Promotional recommendations to reduce the damage of drying and wilting blossom of date palm disorder. Technical Publication of Jihad-e-Agriculture Organization of Kerman Province, Kerman, Iran. P. 16.

Panahi K. (1999). A review on the causes of drying blossom of date palm disorder. Final report of Iran's Dates and Tropical Fruits Research Institute, Ahvaz, Iran. P. 30.

Pezhman H. (2004). Investigation on the effects of drying and wilting blossom of date palm disorder. Final report of Scientific Research Council, Tehran, Iran. P. 125.

Prabhavathi VR, Rajam MV. (2007). Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. *Plant Biotechnology* **24**, 273-282.

Rastogi R, Davies PJ. (1991). Polyamine metabolism in ripening tomato fruit II. Polyamine metabolism and synthesis in relation to enhanced putrescine content and storage life of a/c tomato fruit. *Plant Physiology* **95**(1): 41-45.

Roy P, Niyogi K, Sen Gupta DN, Ghosh B. (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H+-ATPase in salt-tolerant and salt sensitive rice cultivars. *Plant science* **168**(3), 583-591.

Serrano M, Martínez-Romero D, Castillo S, Guillén F, Valero D. (2004). Role of calcium and heat treatments in alleviating physiological changes induced by mechanical damage in plum. *Postharvest Biology and Technology* **34**(2), 155-167.

Shen W, Nada K, Tachibana S. (2000). Involvement of polyamines in the chilling tolerance of cucumber cultivars. *Plant Physiology* **124**(1), 431-439.

Shiozaki S, Ogata T, Horiuchi S. (2000). Endogenous polyamines in the pericarp and seed of the grape berry during development and ripening. *Scientia Horticulturae* **83**(1), 33-41.

Stern RA, Ben-Arie R, Applebaum S, Flaishman M. (2006). Cytokinins increase fruit size of 'Delicious' and 'Golden Delicious' (*Malus domestica*) apple in a warm climate. *The Journal of Horticultural Science & Biotechnology* **18**(1), 51-56.

Tiburcio AF, Altabella T, Borrell A, Masgrau C. (1997). Polyamine metabolism and its regulation. *Physiologia Plantarum* **100**(3), 664-674.

Valero D, Martínez-Romero D, Serrano M. (2002). The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Science & Technology* **13**(6): 228-234.

Ziosi V, Scaramagli S, Bregoli AM, Biondi S, Torrigiani P. (2003). Peach (*Prunus persica* L.) fruit growth and ripening: transcript levels and activity of polyamine biosynthetic enzymes in the mesocarp. *Journal of Plant Physiology* **160**, 1109-1115.

Zokaee Khosroshahi, MR, Esna-Ashari, M. (2008). Effect of putrescine application on post-harvest life and physiology of strawberry, apricot, peach and sweet cherry fruits. *JWSS - Isfahan University of Technology* **12**(45), 219-230.