

Effect of Polyamines, 2, 4-D, Isopropyl Ester and Naphthalene Acetamide on Improving Fruit Yield and Quality of Date (*Phoenix dactylifera* L.)

Kobra Tavakoli and Majid Rahemi*

Horticultural Department, Faculty of Agriculture, Shiraz University, Shiraz, Iran.

(Received: 26 January 2013, Accepted: 4 October 2014)

Abstract

This study was conducted to evaluate the effects of exogenous polyamines, isopropyl ester, 2, 4-D and naphthalene acetamide application on yield and quality of date palm of 'Kabkab' cultivar. The inflorescences sprayed with 2, 4-D isopropylester (0, 10, and 20 mg L⁻¹), naphthalene acetamide (0, 80, and 120 mg L⁻¹), putrescence and spermidine (0, 0.1, and 1.0 mM), three or six weeks after pollination. The highest rate of initial fruit drop (42.2%) was obtained when fruits were treated with naphthalene acetamide at a concentration of 120 mg L⁻¹, which was significantly higher than the control (32.0%). Fruit treated with polyamines had a higher yield than the control. The lowest total soluble solids (TSS) (21.5%) were found in spermidine 0.1 mM and the highest TSS (46%) was found in the control. Polyamines decreased fruit total soluble solids content (TSS) compared with untreated fruits, and also delayed fruit maturity for at least 17 days. In conclusion, polyamine application significantly affected yield, physical and chemical characteristics of date cultivar under the study and it may be recommended to be applied in cultural practices to enhance production and improve the fruit quality of date trees orchards.

Keywords: growth regulators, putrescence, spermidine.

Introduction

Date palm (*Phoenix dactylifera* L.), a monocotyledonous and dioecious species belongs to the Palmaceae family, is widely cultivated in arid regions of the Middle East and North Africa (Al-Khayri, 2001); so it is considered as one of the major agricultural crops of the Near East region, where about 90% of the world's date production is annually harvested (Ahmed, 1999). In many countries of this region, the date palm plays an important economical role and is considered as a generating source, with the potential of staple food

qualities as its fruit is enriched with a higher mineral content (Al-Shahib, 2003). Iran is considered one of the largest producers of dates with a production of about one million tons annually, which is about 15% of total world production. As the main export crops of Iran, they are distributed in warm climate areas, especially in south, southwest and southeast areas in this country. The main area of date cultivation in Iran is Dashtestan region in Boushehr province and the main cultivar is 'Kabkab'.

Nowadays, the use of plant growth regulators in crop production is growing dramatically, and several studies have been done in various fields of plant sciences

* Corresponding author
Email: rahemi@shirazu.ac.ir

(Asna-ashari *et al.*, 2006). Exogenous auxin application may improve size and the pulp ratio to seeds of fruits, and also may lead to a delay in fruit maturation and ripening (Ronca *et al.*, 1998). However, the application of auxins at higher concentrations has an adverse effect on fruit ripening and may totally prevent the ripening of date palm fruits (Stewart and Hield, 1950). Exogenous auxin (NAA) treatment may cause fruit thinning and increases fruit size and fruit set, and also improves fruit quality and yield on two apple fruits (Kosina, 2006) Furthermore, auxin improves fruit yield in kiwi fruits (Bregoli *et al.*, 2010). Previous studies on date palms have shown that application of auxins increased fruit size, the ratio of pulp to seed, and fruit quality (Aplbaum, 1986; Bahrami, 1997; Izadi, 2008; and Mohammadi *et al.*, 2008).

Polyamines are known as a new group of plant growth regulators that can cause growth stimulation by increasing their biosynthesis in the plant tissues (Arias *et al.*, 2005). The presence of polyamines in plant tissues shows that they may have a key role in the regulation of plant growth and development. The effect of exogenous polyamine application has been studied on different fruit trees including avocado (Aplbaum, 1986), mango (Malik and Singh, 2000; Malik and Singh, 2006; Malik and Singh, 2004), strawberry (Asna-ashari *et al.*, 2006), sweet orange (Saleem *et al.*, 2008) and lychee (Stewart and Hield, 1950). Polyamines reduce ethylene production, probably by preventing enzymatic conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Aplbaum and Ickson, 1983), which results in reducing growth and delay in fruit ripening (Aplbaum, 1986). Studies on avocado (Aplbaum and Ickson, 1983; Winer and Apelbaum, 1986), mango (Malik and Singh, 2004; Malik and Singh, 2006; Malik and Singh, 2000) and strawberry revealed the effects of polyamines on preventing ethylene synthesis and reduction of total soluble solid content (TSS) of the fruits. Arginine

decarboxylase is a key enzyme involved in the synthesis of polyamines. Transcripts of polyamines biosynthesis controlling genes code arginine decarboxylase under stress (Ziosi *et al.*, 2009). Studies on mango (Malik and Singh, 2000), apple (Kosina, 2006), pear (Franco- Mora *et al.*, 2005), sweet orange (Saleem *et al.*, 2008) and avocado (Kushad *et al.*, 1988) have shown that exogenous polyamine application reduces fruit abscission and increases fruit yield. However, information on the effect of plant growth regulators application during fruit development is still scarce. The aim of this experiment was to clarify the role of plant hormones in fruit growth and development, and also the quality and quantity of date fruits of the 'Kabkab' cultivar.

Material and Methods

Plant material

The experiment was conducted on date palm 'Kabkab', at the Date Palm Research Center of Iran, Bushehr, Iran, during the 2010 and 2011 growing seasons. Uniform size fruits were selected for the experiment.

Plant growth regulators with different concentration of spermidine (0, 0.1 and 1.0 mM), putrescine (0, 0.1 and 1.0 mM), 2,4-diisopropyl ester (0, 10, and 20 mg L⁻¹), naphthalene acetamide (0, 10, and 20 mg L⁻¹), and control (distilled water) were sprayed on three flower clusters in different directions on the trees, three and six weeks after pollination.

Fruits growth and quality evaluation

Fruit growth, length and diameter of ten fruits were measured by a digital caliper. Ten fruits were weighed and mean fruit weight was recorded. Fruit length and diameter were weighted, separately.

Total soluble solids (TSS) and fruit juice pH were measured by a digital refractometer and a digital pH meter, respectively. The number of ripped fruits per cluster was counted and ripe fruit percentage was calculated.

Statistical analyses

The experimental design was a randomized complete block design (RCBD) which was repeated twice, and each time with four replications per treatment. The data were analysed by MSTAT-c software and the means were compared using Duncan's Multiple Range test at $P \leq 1\%$.

Results

The effect of different plant growth regulators on fruit weight is shown in Table 1. The highest fruit weight, 128.8 g, was found in 120 mg L⁻¹ naphthalene acetamide, which was significantly higher than the control treatment (112.8 g). The lowest fruit weight, 109.6 g, was found in 0.1 mM putrescine treatment, which was significantly lower than the control treatment.

The highest fruit length (38.7 mm) was obtained with 120 mg L⁻¹ naphthalene acetamide treatment, which was significantly higher than the control (31.8 mm). The lowest fruit length, (26.1 mm), was found in the putrescine 1.0 mM, which was significantly lower than the control treatment (Table 1).

The effects of the treatments on the diameters of the fruits are shown in Table 1. These treatments significantly increased fruit diameter compared to the control treatment (22 mm). Although there was no significant difference between the fruit diameter of treated trees, the fruit diameters in 120 mg L⁻¹ naphthalene acetamide (23.9 mm) and 80 mg L⁻¹ (24.6 mm) treatments were 11.8% and 8.6% higher than the control treatment, respectively.

Table 1. Effects of exogenous application of different growth regulators on the characteristics of date palm fruit growth.

Treatments	Concentration	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)
Control	0	112.8 c [†]	31.8 cb	22 a
Spermidine	0.1 mM	121 a	36.4 b	23.2 a
Spermidine	1.0 mM	119.b	26.1 c	22 a
Putrescine	0.1 mM	109.6c	31.1 bc	21.3 a
Putrescine	1.0 mM	115 c	34.1bc	22.6 a
2,4-D	10 mg L ⁻¹	125 c	35.1 abc	22.2 a
2,4-D	20 mg L ⁻¹	126.1 ab	35.2 abc	23.5 a
NAD	80 mg L ⁻¹	128.5 a	35.75 ab	24.6 a
NAD	120 mg L ⁻¹	128.8 a	38.7 a	23.92 a

[†] Means with the same letters did not show a significant difference in accordance to Duncan's multiple range test, at $P \leq 0.01$.

Total fruit weight per fruit cluster was significantly lower in the control treatment (7.5 kg). The highest total fruit weight per fruit cluster (10.8 kg) was obtained in 1.0 mM spermidine, which was significantly higher than the other treatments (Table 2). Although applications of 0.1 and 1.0 mM putrescine increased fruit weight per cluster, the differences were not significant compared to the control treatment (Table 2).

The highest rate of initial fruit drop (42.2%) was found in the 120 mg L⁻¹

naphthalene acetamide, which was significantly higher than the control treatment (32.0%). The lowest rate of initial fruit drop was found in the 1.0 mM and 0.1 mM putrescine treatments; however, there were no significant differences between the two treatments and the control treatment (Table 2). Secondary fruit drop was significantly reduced in 0.1 mM and 1.0 mM putrescine treatments (Table 2).

Table 2. Effects of exogenous applications of polyamines and auxins on date palm fruit drops and yield.

Treatments	Concentration	Initial Fruit Drop (%)	Secondary Fruit Drop (%)	Yield (kg cluster ⁻¹)
Control	0	32 bc [†]	25 a	7.5 d
Spermidine	0.1 mM	11 c	11 c	10.4 a
Spermidine	1.0 mM	5.2 c	5.2 c	10.8 a
Putrescine	0.1 mM	2.5 c	2.5 c	9.9 b
Putrescine	1.0 mM	2.5 c	2.5 c	10.4 a
2,4-D	10 mg L ⁻¹	35 b	17.5 b	8.5 ab
2,4-D	20 mg L ⁻¹	34 b	16 b	9.01 ab
NAD	80 mg L ⁻¹	37.9 b	18.5 b	9.03 ab
NAD	120 mg L ⁻¹	42.4 a	20 a	9 c

[†] Means with the same letters did not show a significant difference in accordance with Duncan's multiple range test, at $P \leq 0.01$.

The highest rate of fruit drop was observed in the control treatment (25%), which was significantly higher than other treatments. Total soluble solid content (TSS) of the fruits was significantly different in the experimental treatments (Table 3). The highest TSS value was found in the control treatment (62.8%). The lowest TSS content (34.0%) was found in spermidine 0.1 mM, which was significantly lower than the other treatments (Table 3). Table 3 shows the effect of experimental treatments on fruit juice pH. Fruit pH was significantly higher in the

control (7.4) and 2, 4-D (7.5) treatments. Effects of polyamines on reducing fruit juice pH were higher than the auxin treatments and the lowest fruit juice pH (5.6) was found in spermidine 1.0 mM. The number of ripened fruits was significantly higher in the control (95%) and naphthalene acetamide 120 mg L⁻¹ (97%) (Table 3). The lowest number of ripe fruits was found in spermidine 0.1 mM (75%), which was significantly lower than the control treatment. Spermidine 0.1 mM had a delay in fruit ripening for 17 days in comparison to the control treatment (Table 3).

Table 3. Effects of exogenous application of polyamines and auxins on date palm fruit quality indices and fruit ripening delay.

Treatments	Concentration	TSS (%)	pH	Delay in Fruit ripening (Day)
Control	0	62.8 a [†]	7.4 a	-
Spermidine	0.1 mM	39 b	5.5 c	17 b
Spermidine	1.0 mM	34 b	6.2 b	14 ab
Putrescine	0.1 mM	40.1 b	6.3 b	12 b
Putrescine	1.0 mM	38.2 b	5.6 c	10 b
2,4-D	10 mg L ⁻¹	57 a	7 ab	8 c
2,4-D	20 mg L ⁻¹	56 a	7 ab	8 b
NAD	80 mg L ⁻¹	56.3 a	7.3 ab	9 b
NAD	120 mg L ⁻¹	58.7 a	7 ab	9 b

[†] Means with the same letters did not show a significant difference in accordance to Duncan's multiple range test, at $P \leq 0.01$.

Discussion

There were no significant differences between the results of the two years of this study. The effects of experimental treatments on fruit growth and quality were different. Fruit growth indices involving mean fruit weight, fruit length and fruit diameter, were significantly higher in auxin treated fruits.

The results of this investigation showed that auxins are more effective than polyamines in increasing the growth of date palm fruits. Experimental treatments had no significant effect on fruit diameter. However, the highest fruit weight, fruit length and diameter were found in 120 mg L⁻¹ naphthalene acetamide treatment (Table 1). The results are in accordance to the results of Bahrami, 1997; Izadi, 2008; and Mohammadi *et al.*, 2008.

In this study, polyamines slightly increased fruit growth indices, i.e., fruit length and weight. Previous researches on strawberry (Asna-ashari *et al.*, 2006), sweet orange (Saleem *et al.*, 2008), and lychee (Stern and Gazit, 2000) also showed the effect of polyamines on increasing fruit growth indices. Bais and Ravishankar (2002) stated that polyamines are a new group of plant growth regulators and may act as secondary signal transductions. They control important physiological processes in plants such as embryogenesis, cell division, morphogenesis and plant development (Bais and Ravishankar, 2002).

The highest rate of secondary fruit drop was found in the control treatment (25%), which was significantly higher than other treatments (Table 2). Putrescine treatments significantly reduced secondary fruit drop (Table 2). Taghipour and Rahemi (2009) and Mohammadi *et al.* (2008) also found the same results on using auxins on date palm. It has been reported that the application of auxins may change hormonal balance in fruits and induce ethylene evocation which leads to fruit drop (Bregoli *et al.*, 2010). Generally, treatments that

induce fruit drop and fruit number reduces the competition between the fruits for the photosynthesis assimilates, which gradually lead to an increase in fruit growth. Hence, the effect of naphthalene acetamide on increasing date palm fruit growth indices may be related to inducing fruit drop. Ravankar (2009) reported the same results for lime fruit.

In this study, polyamine application reduced date palm fruit drop. Studies of Malik *et al.* (2006) on mango, Kosina (2006) on apple, Franco (2005) on pear, Malik and Singh (2000) on mango, Saleem (2008) on sweet orange, and Kushad (1988) on avocado also showed that exogenous polyamine application reduced fruit drop.

Polyamines reduced ethylene production, probably by preventing enzymatic conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Aplbaum and ICKEKSON, 1983); and so their application indirectly reduced fruit drop (Aplbaum, 1986). It has been stated that effects of exogenous polyamines on preventing fruit drop and increasing yield depends on time and concentration of the treatments. In this study, polyamines applied on the fruits at two different times during the active growth of the fruits, prevented fruit drop and increased fruit yield. The highest fruit yield (10.8 kg cluster⁻¹) was obtained in spermidine 1 mM, which was significantly higher than other treatments (Table 2). Studies of Malik *et al.* (2006, 2000) showed that exogenous polyamines application increased mango fruit growth and yield.

The highest TSS value was found in the control treatment (62.8%). TSS was significantly lower in spermidine 0.1 mM (34%) (Table 2). Malik *et al.* (2000, 2006) and Asna-ashari *et al.* (2006) reported that exogenous polyamine application may reduce the TSS of fruits. TSS was reduced in auxin-treated fruits. Previous research on date palm has shown that exogenous auxin application may reduce the TSS of

fruits (Izadi, 2008; Bahrami, 1997; Mohammadi *et al.*, 2008). Exogenous auxins and cytokinins increase proteins biosynthesis via increasing RNA transcription and RNA polymerase activity. In addition, auxins induce cell elongation and increase cell volume, which is followed by a water influx increase (Alcazar *et al.*, 2006; Malik and Singh, 2000). Hence, reduced TSS after auxin application may be related to a dilution effect due to higher water absorption.

Different treatments had different effects on date palm fruit ripening. The highest number of ripped fruits was found in 120 mg L⁻¹ naphthalene acetamide (97%), and the lowest number was found in 0.1 mM spermidine (80%). As Aylward (1984) stated, this result may be due to the effect of polyamines on reducing ethylene biosynthesis by reducing the activity of ACC-decarboxylase. The result suggested

that free polyamines may act as anti-senescence factors in the plants.

Exogenous auxins application delayed date palm fruit ripening (Table 2). Bahrami (1997), Izadi (2008) and Mohammadi *et al.* (2008) also reported that exogenous auxins application delayed date palm fruit ripening. Alcazar *et al.* (2006) stated that there is an induced delay in the ripening of fruits after exogenous auxin application, which is probably due to a reduction of acetaldehyde biosynthesis, which has shown that production of some acetaldehyde is critical for fruit ripening (Arias *et al.*, 2005). This study can lead to the conclusion that spraying of polyamines significantly affects the yield, physical and chemical characteristics of 'Kabkab' date cultivar, and it is recommended that it is applied in cultural practices to enhance production and improve fruit quality of date tree orchards.

References

1. Abu-Kpawoh, J.C., Y.F., Xi, Y.Z., Zhang and Y.F., Jin. 2002. Polyamine accumulation following Hot-water dips influence chilling injury and decay in friar plum fruit. *Food Chem. Toxic.* 67(7):2649-2653.
2. Asna-ashari, M., Zakayie Khosro-Shahi, M. R. and Ershadi, M. 2006. Effect of exogenous putrescine on postharvest life of strawberry. *J. Res. Agr. Sci.* 6:15-25 (in Farsi).
3. Al-Khayri J.M. 2001. Optimization of biotin and thiamine requirements for somatic embryogenesis of date palm (*Phoenix dactylifera* L.). *In Vitro Cell. Dev. Biol. Plant* 37:453-456.
4. Al-Shahib, W. and R.J. Marshall. 2003. The fruit of the date palm: it's possible use as the best food for the future? *Int. J. Food Sci. Nutr.* 54: 247-59.
5. Ahmed, A.A., 1999. Date palm postharvest processing technology in Libya. Regional Workshop on Date Palm Postharvest Processing and Technology in Iran.
6. Alcazar, R, F. Marco, J.C. Cuevas, M. Patron, A. Ferrand, P. Carrasco. A. F. Tiburcio and T. Altabella. 2006. Involvement of polyamines in plant response to abiotic stress. *Biotechnol. Lett.* 28 1867-1876.
7. Aylward, A, C. Burgeon, J. Andrew, M. Lieberman, R. benarie and A. Mattod. 1981. Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplast. *Plant Physiol.* 68:453-456.
8. Aylward, A and I. Ickson. 1983. Applied polyamines inhibit macromolecule synthesis in plant tissue. In: *Advances in Polyamine Research*. Bacharach, U., A. Kaye and R. Chayen. Ravan Press, New York, pp: 437-442.
9. Aylward, A. 1986. Polyamine involment in the development and ripening of avocado fruit. *Acta Hort.* 179:779-785.
10. Arias, M., J. Carbonell and M. Agusti. 2005. Endogenous free polyamines and their role in fruit set of low and high parthenocarpic ability citrus cultivars, *Plant Physiol.* 126(8):845-853.
11. Bahrami, H.R. 1997. Evaluating the effects of naphthaleneacetic acid, naphthalene acetamid, and 2, 4-diisopropylester on fruit size, yield, quality, and ripening time of date palm 'Shahani'. M.Sc. thesis in Horticultural Science,

- Islamic Azad University, Branch Jahrom, Iran. (In Farsi).
12. Bregoli, A, M. C. Fabbroni, F. Costa, S. Stella, V. Ziosi and G. Costa. 2010. Kiwifruit growth control by synthetic auxin 3, 5, 6-TPA. *J. Hort. Sci.* 727.
 13. Bais, H.P. and G.A. Ravishankar. 2002. Role of polyamines in the ontogeny of plants and their biotechnological applications. *Plant Cell Tiss. Organ Cult.* 69:1-34.
 14. Franco-Mora, O., K. Tanabe, F. Tamura and A. Itai. 2005. Effects of putrescine application on fruit set in Housi Japanese pear. *Sci. Hort.* 104: 265-273.
 15. Izadi, M. 2008. Effects of benzyladenine, NAA, and 2, 4-Diisopropylester on yield and quality of date palm 'Kabkab'. M.Sc. thesis in horticultural science, Shiraz University, Iran. (In Farsi).
 16. Kushad, M.M., G. Yelenosky and R. Knight. 1988. Inter relationship of polyamine and ethylene biosynthesis during avocado fruit development and ripening. *Plant Physiol.* 87:463-467.
 17. Kosina, G. 2006. Response of two apple cultivars to chemical fruit thinning. *Acta Hort.* 774p.
 18. Malik, A.U. and Z. Singh. 2004. Endogenous free polyamines of mangos in relation to development and ripening. *J. Amer. Soc. Hort. Sci.* 129:280-286.
 19. Malik, A.U. and Z. Singh. 2006. Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *Hort Sci.* 110:167-174.
 20. Malik, A.U. and Z. Singh. 2000. Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *J. Hort. Sci.* 70:271-277.
 21. Ronca, F., H. Arbiza and A. Albella *et al.* 1998. Synthetic Auxin evaluation on fruit size and yield in Lisbon Type lemon. *Acta Hort.* 463:405-412.
 22. Mohammadi, A., A. Aboutalebi, H. Hasanzadeh, and M. Mohammadi. 2008. Evaluating the effects of plant growth regulators on quality and yield of date palm 'Shahani'. *J. Res. Agr. Sci.* 4:204-211. (In Farsi).
 23. Saleem, B. A, A. U. Malik, R. Anwar and M. Farooq. 2008. Exogenous application of polyamines improves fruit set, yield and quality of sweet oranges. *Acta Hort.* 774-779.
 24. Stewart, W.S. and H.Z. Hield. 1950. Effects of 2, 4-D and 2,4-T on fruit drop, fruit production and drop of lemon Trees. *Proc. Amer. Soc. Hort. Sci.* 55:71-163.
 25. Stern, A.R. and S. Gazit. 2000. Application of the polyamine putrescine increased yield of "Maurititu" Litchi (*Litchi chinensis* Sonn). *J. Hort. Sci. Biotechnol.* 75:612-614.
 26. Singh, Z. and L. Singh. 1995. Increased fruit set and retention in mango with exogenous application of polyamines. *J. Hort. Sci.*, 70, 271-277.18, 59-61.
 27. Sood, S. and P.K. Nagar. 2008. Postharvest alteration in polyamines and ethylene in two diverse rose species. *Acta Physiol. Plant* 30:243-248.
 28. Taghipour, L. and M. Rahemi. 2009. Effects of plant growth regulators on quality and yield of apricot. *J. Hort. Sci.* 23:78-84. (In Farsi).
 29. Takahashi, T. and J.I. Kakehi. 2010. Polyamine: Ubiquitous polycations with unique roles in growth and stress responses, *Ann. Bot.* 105:1-6.
 30. Wing, G., Q. Xu. Shii and J. HU. 2006. Exogenous polyamines enhance toleranc of nymphoides peltatum. *J. Plant Phisiol.* Doi: 10.1016/jplph. 2006. 06. 003.
 31. Wilkins, M.B. 1946. *The Physiology of Plant Growth and Development.* Mcgrow-Hill Book Company. London. 695 p.
 32. Winer, L. and A. Apelbaum. 1986. Involvement of polyamines in the development and ripening of avocado fruits. *J. Plant Physiol.* 126, 223-233.
 33. Ziosi, V., A.M. Bregoli, F. Fregola, G. Costa and P. Torrigiani. 2009. Jasmonate-induced ripening delay is associated with up-regulation of polyamins level in peach fruit. *J. Plant Physiol.* 166:938-946.