Effect of foliar application of naphthalene acetic acid and plant thinning on sugar contents of melon (*Cucumis melo*) fruit cv. Khatooni

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Abstract

Total soluble solid (TSS) content is commercially used as fruit quality index because of its high positive correlation with sugar content. In the present study, The effect of foliar application of naphthalene acetic acid (NAA) with different concentrations (0, 25, 50, and 100 $mg.L^{-1}$) at 4 true leaf and fruit set stages and plant training (pruning and thinning) on TSS and soluble sugar (i.e. sucrose, glucose and fructose) contents of fruit were evaluated in melon (*Cucumis melo* L.) cv. Khatooni. Results showed that NAA treatments significantly increased TSS and soluble sugar contents. The highest TSS and soluble sugar contents were observed in 100 $mg.L^{-1}$ NAA. In contrast, TSS and soluble sugar contents were decreased significantly by plant thinning. In mature fruits, an obvious gradient of TSS and soluble sugar contents was detected, ascending from pedicel to middle and umbilicus part of mesocarp. Also sucrose was dominant sugar in ripening fruit. Results of this work suggest that application of 100 $mg.L^{-1}$ NAA at fruit set could be used to increase TSS and soluble sugar contents of melon cv. Khatooni.

Keywords: foliar application; thinning, mesocarp, total soluble solids

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Introduction

Melon is one of the economically important and widely cultivated fruit crops in the world. Iran is the third largest melon-producing country in the world (FAOSTAD, 2010) with great potential to produce and export high quality melons. Fruit sweetness is the major determinant of fruit quality, and assessing the marketing value in melons reflects the concentration of the three major soluble sugars, i.e. sucrose, glucose, and fructose, in the fruit flesh (Li et al., 2006). Total soluble solid content is commercially used as fruit quality index because it has a high positive correlation with sugar content. More than 97% of the total soluble solids in melon fruits are soluble sugars, of which sucrose is the predominant sugar found in the ripened fruits, accounting for 50% of the total soluble sugars (Pharr, 1994). Also, relative contents of sucrose, glucose and fructose change as the melon fruits mature. In addition, Mizuno et al. (1971) showed that

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different parts of the fruit flesh of muskmelons have different sugar compositions. In proviso, we studied the sugar accumulation in two Iranian melon genotypes 'Zard Jalali' and 'Suski Sabz'. Our results showed that sucrose is the predominant sugar present in the ripened fruits of melon, accounting for 50% of the soluble sugars. Also there is a significant difference in sugar accumulation (i.e. glucose, fructose, and sucrose) among mesocarp parts, ascending from pedicel to middle and umbilicus parts (Barzegar et al. 2011).

Biomass and carbohydrate contents of fruit are manipulated by agronomic practices that increase assimilates partitioning to fruit in a number of crops. For example, hand and chemical thinning of fruits are common in the apple (Basak 2002), citrus (Stover et al., 2001), and stone fruit (Byers et al., 2003) industries to increase fruit size and TSS.

To provide conditions for maximum yield production of sweet and high-quality fruits, pruning and thinning offer the opportunity to adjust the fruit load of each individual plant according to its vegetative vigor (Papadopoulos, 1994). Experienced Iranian farmers, using pruning (cutting the main stem and maintaining two lateral branches) and thinning (removing all female flowers produced before and after 6^{th} – 8^{th} node from the base of each lateral branch) practices, traditionally keep only one fruit on one of 6^{th}, 7^{th} or 8^{th} node of each lateral branch, i.e. keep two fruits per plant. They believe that these practices are necessary to increase average fruit weight, yield and quality of melon (Barzegar et al., 2013). Now, based on using new methods and technology, we found that fruits which ^{13}C-labelling are set and remained on 6^{th} - 8^{th} nodes of each lateral stems have the highest potential to absorb assimilate from different leaves located on both sides of stem (Barzegar et al., 2013). These agronomic practices require too much time and labor for the farmers. So, practices to produce large and high-quality fruits in this cultivar without thinning are needed.

Plant growth regulators can also be used to influence source–sink balance in melons. The use of plant growth regulators such as NAA, GA_3, and indole-3-butyric acid (IBA) by many researchers has been shown. Naphthalene acetic acid (NAA), a synthetic growth regulator is known to affect the growth, development, and other physiological and biochemical processes of plants (Ravi Kher et al., 2005). Kano (2002) studied the influence of 4-chlorophenoxy acetic acid (4-CPA) on sucrose accumulation and cell size of melon fruits (*Cucumis melo* L.) and reported that sucrose content in the 4-CPA-treated fruits was greater than that of the control fruits.

The application of 100 mgL^{-1} NAA, 14 days after blooming increased total soluble solid content and improved epicarp and mesocarp thickness of muskmelon cv. Edisto 47 (Mata and Natera, 2009). In contrast, Ouzounidou et al. (2006) reported that under the application of GA_3, the sugars (fructose, glucose, and sucrose) and the soluble solids of melon (*C. melo*) cv. Galia fruits remained unchanged compared to the control.

With this background, the aim of the present work is to study the effects of naphthalene acetic acid (NAA) on sugar content of melon (Khatooni) and to compare this with the thinning practice.

Material and methods

Plant material and culture

A field experiment was conducted during June to September 2013 at Research Farm of College of Agriculture, University of Zanjan, to study the effect of naphthalene acetic acid (NAA) on sugar content of different parts of commercial Iranian melon, 'Khatooni' (*Cucumis melo* L. Inodorous group).

'Khatooni' cultivar has yellow-green netted skin color with chimeric stripes, oblong in shape, and large-sized fruit weighing approximately 3-4 kg per fruit at harvest time (Fig. I). The seeds for experiment were collected from Iranian farmers in Mashhad. Fertilizer was delivered as a pre-plant base comprising 80 kg N/ha, 50 kg p/ha and 80 kg K/ha. Irrigation was subjectively delivered to meet the crop requirements. The seeds were sown at 3-4 cm depth, 50 cm spacing in row with 200 cm between rows in farm. After pruning (cutting the main stem), Plants were sprayed with NAA at 0 (control), 25, 50, and 100 mg/l at 4-true-leaf and

Fig.I. Iranian melon cultivar studied "Khatooni"

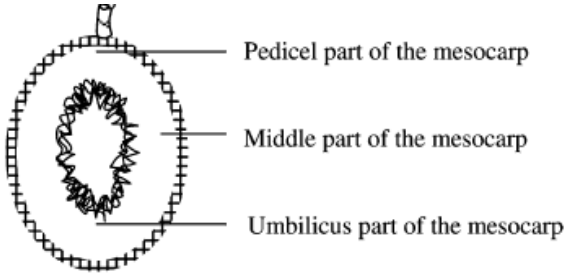

Fig. II. Sampled parts of fruit tissue (Zhang and Li, 2005)

fruit set stages. Also, after pruning, some plants were trained to two lateral branches and only 2 fruits per plant were allowed to set (i.e. one fruit per lateral branch) on similar node (7th node position). This method of thinning-pruning procedure is indigenous to Iran context which is traditionally practiced by Iranian melon producers.

Total soluble solids

The fruits were harvested when color changed from green to yellow and appearance of the netted pattern. Total soluble solids (TSS) of fruit mesocarp tissues were determined. Each sampled fruit was divided in three edible parts: pedicle (mesocarp close to peduncle), middle part of mesocarp, and distal part (mesocarp close to umbilicus) (Fig. II). For TSS assessment, a 22 mm diameter core of each mesocarp tissue was extracted. For each core 5 mm of outer rind and green inedible tissue, and inner seeds, were removed and juice was extracted from the remaining edible white mesocarpe tissue. Total soluble solid of the juice was determined using a hand refractometer (Younglin, Rep. of Korea). The TSS was expressed in °Brix.

Analysis of soluble sugar contents

Harvested fruit were brought to the laboratory and sampled as follows. Samples were immediately frozen in liquid nitrogen, stored at -80 °C. Samples of mesocarp tissues were homogenized for 5 min in 5 volumes of extracted solution [ethanol (E): chloroform (C): water (W) = 12:5:3, L L−1]. Water and chloroform were then added to bring the final E:C:W ratio to 10:6:5. Subsequent separation of the chloroform layer allowed removal of lipids and pigments. The remaining aqueous-alcohol phase was evaporated to dryness in vacuum at 50 °C and re-dissolved in 1 ml distilled water. Analysis of

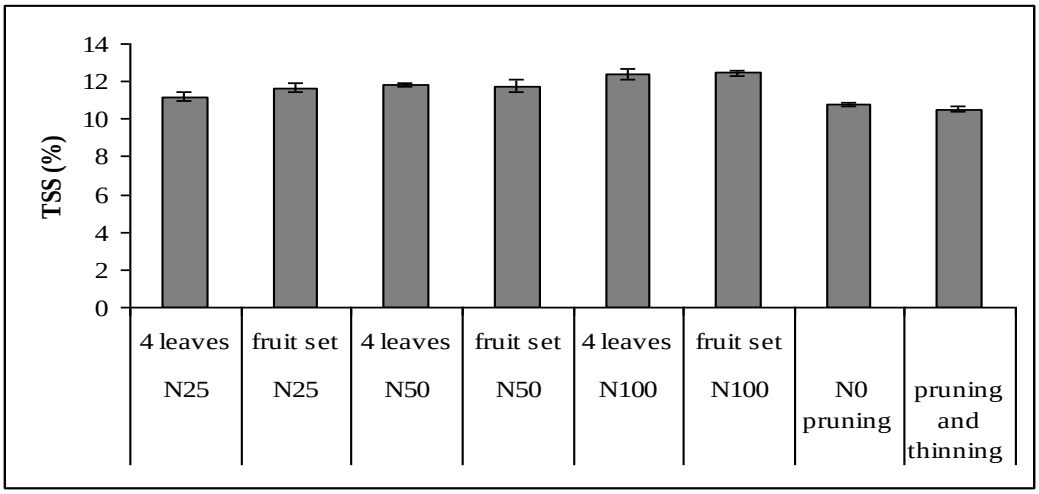

Fig. III. Effect of different concentrations of NAA (N) at two foliar application times and plant tillage practise on TSS contents of mature 'Khatooni' melon fruits; data correspond to means of measurements ±SE. (P< 0.05).

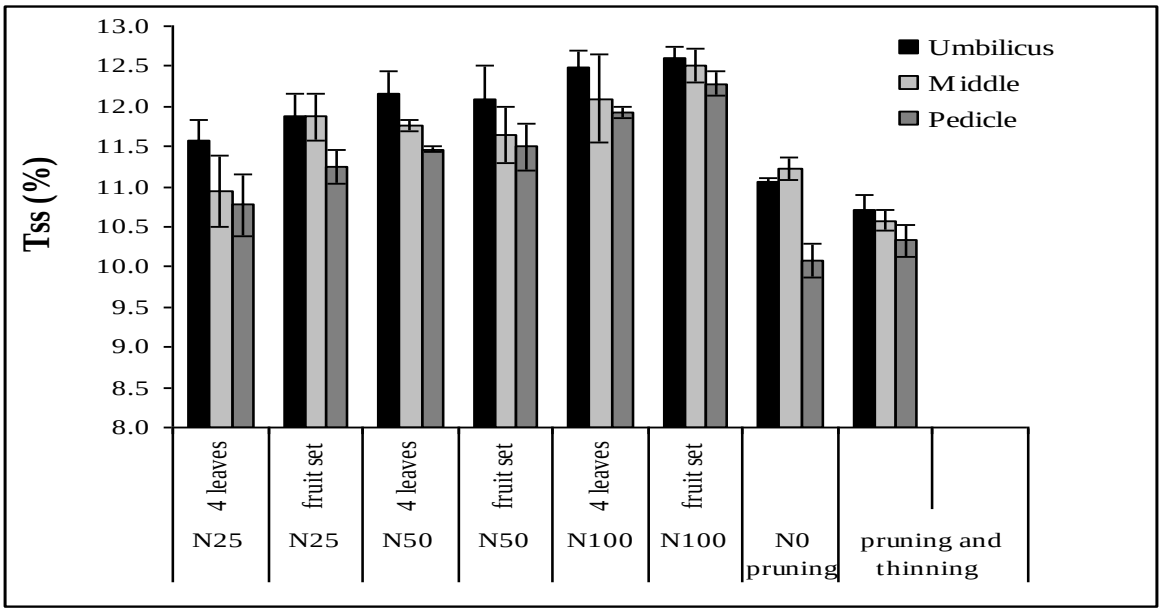

Fig. IV. Comparison of the effect of different concentrations of NAA (N) at two foliar application times and plant tillage practise on TSS contents in different mesocarp parts of mature fruits in 'Khatooni' melon; data correspond to means of measurements ±SE. (P< 0.01).

sucrose and reducing sugars (i.e. glucose and fructose) was performed by high performance liquid chromatography (KNAURE, Germany) with an NH2 column at 30 °C using 65% acetonitrile solution (1 mL min−1) as a mobile phase and a refractive index detector (RI). Peaks were quantified by using corresponding analytical software. Sweetness index was calculated on the basis of relative values being rated as 50 (Komatsu et al., 1999).

Results

TSS in different parts of mesocarp

The total soluble solid is considered as important quality parameter in muskmelon. The data on TSS in mature fruits indicated significant differences between mesocarp parts and treatments. Among the treatments, maximum total soluble solids content (12.47 ± 0.156) was recorded in 100 ppm NAA (spray at fruit set) followed by 100 ppm NAA (spray at 4-true-leaf stage). The lowest TSS content (10.54 ± 0.156) was obtained in thinning treatment which was significantly lower compared to all the other treatments except control (pruning) (Figs. III and IV).

In three mesocarp parts of mature fruits, maximum total soluble solids content was recorded in umbilicus part, followed in middle and pedicel parts (Fig. IV). As the results showed the TSS content of the mesocarp tissues increased with an increase in the concentration of NAA (Fig. III).

Soluble sugar contents

The sucrose and reducing sugar (i.e. glucose and fructose) contents were measured in mature fruit. The results showed that application of NAA increased the reducing sugar content of melon fruit at harvest and significant differences were observed between treatments (Fig. V). Among the treatments, the maximum contents of sucrose (5 % per ml juice) and fructose (2.23 % per ml juice) was obtained in NAA 100 ppm (spray at fruit set) which had significant difference with all other treatments (Fig. V). Also, the highest amount of glucose (1.4 % per ml juice) was found in pruning plants. In mature fruits, sucrose was the most abundant sugar followed by fructose and glucose (Fig. V).

Discussion

Sweetness (i.e. sugar content) is the most important factor determining the eating quality

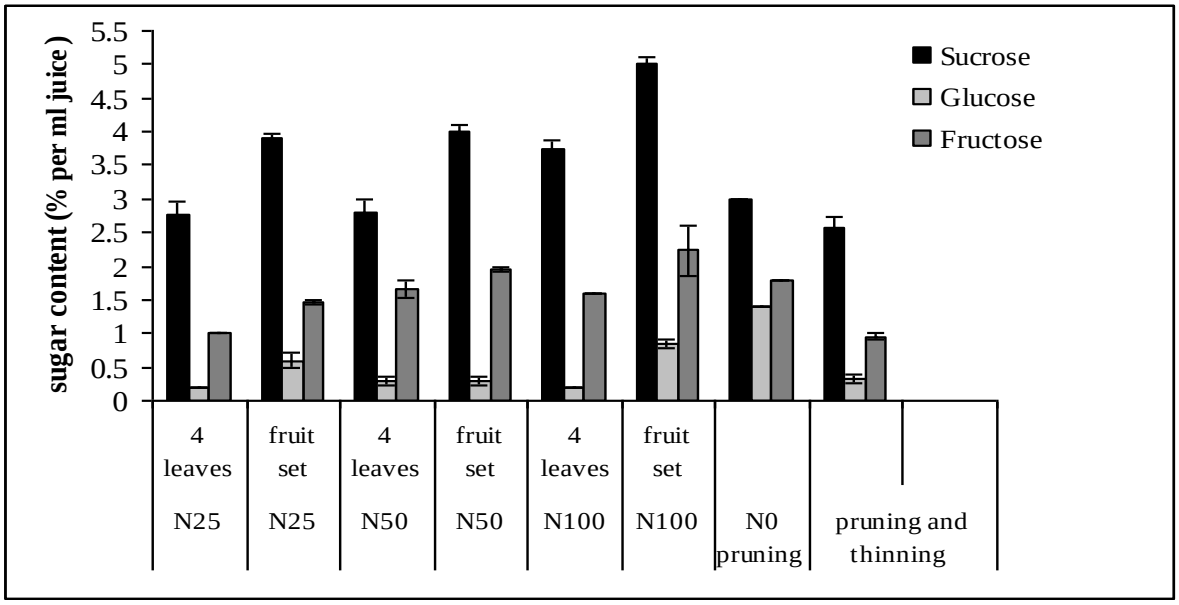

Fig. V. Individual soluble sugar contents in mesocarp tissue of Iranian melon cultivar 'Khatooni' as affected with different concentrations of NAA (N) at two foliar application times and plant tillage

of melon fruits (Mutton et al., 1981). Effects of foliar application of NAA on soluble sugar contents at harvested stage have been studied in Iranian melon. In the present study, we analyzed soluble sugar contents in "Khatooni" fruits at three edible parts of fruit mesocarp i.e. pedicle, middle part of mesocarp and umbilicus. Our results indicate that there is a significant ($P \leq 0.01$) difference in TSS among mesocarp parts, ascending from pedicel to middle and umbilicus parts (Fig. III A and D). This result is consistent with the finding of Mizuno et al. (1971) who showed that different parts of the flesh of muskmelon have different sugar compositions. We showed that the amount of sucrose in mesocarp tissues of mature melon fruits was markedly higher than glucose and fructose (Table 1). Also as reported by many researchers, sucrose is the predominant sugar present in the ripened fruits of melon, accounting for 50% of the soluble sugars (Lester and Dunlap, 1985; Pharr, 1994; Lester et al., 2001). In contrast to our results, Zhang and Li (2004) found in the Chinese melons that fructose was the predominant sugar. Also, relatively low levels of sucrose in a few orange-fleshed cantaloupes have been reported (Lingle and Dunlap, 1987; Stepansky et al., 1999). Although source leaf photosynthetic activity and fruit position on the stem play a role in determining the assimilate supply to melon fruit (Barzegar et al. 2013), the accumulation of sucrose appears to be controlled by the metabolism of carbohydrates in the sink (i.e. fruits) itself (Lester et al., 2001). Chrost and Schmitz (1996) suggested that the accumulated sucrose is synthesized from available glucose and fructose. On the other hand, the changes in the sugar composition of developing fruits corresponded to changes in the activity of sucrose metabolizing enzymes (Lingle and Dunlap, 1987). They showed that during early period of fruit development, acid and neutral invertase (AI) activities were high, and sucrose phosphate synthase (SPS) activity was low. As fruits matured, relative increase in sucrose content occurred associated with decline in invertase activity and increase in SPS activity (Lingle and Dunlap, 1987). Hubbard et al. (1991) showed that sucrose accumulation in melon fruits was characterized by a developmental increase in SPS activity, in addition to the developmental loss of AI activity. Lester et al. (2001) confirmed the importance of the loss in AI activity and the increase in SPS activity in two sweet melon cultivars and emphasized particularly the necessity for SPS activity to be higher than that of AI. Also, Burger et al. (2007) indicated that sucrose accumulation in the developing fruits of

Cucumis melo began only when AI activity declined to less than an experimentally determined threshold value, and continued until removal of the fruit from the plant. In addition, the activities of sucrose phosphate synthase, sucrose synthase, and neutral invertase were all positively correlated with sucrose accumulation among the genotypes. Yativ et al. (2010) reported that sugar composition in watermelon, as in all cucurbit fruits, includes sucrose, fructose and glucose. They indicated that, within the genus *Citrullus*, there are genotypes that accumulate a high percentage of sucrose in fruits, while others accumulate glucose and fructose.

Significant differences were observed in TSS content of fruits due to different plant growth regulators. Maximum TSS was recorded in 100 ppm NAA (spray at fruit set). The TSS content of the fruits increased with an increase in the concentration of NAA. The increase in TSS content by PGR's could be due to diversion of more solids towards developing fruits and might also have enhanced the conversion of complex polysaccharides into simple sugars (Ravi Kher et al., 2005). Also the increase in TSS could be attributed to the enhanced photosynthetic efficiency of the leaves and a possible increase in translocation of assimilates into the fruits in response to hormonal stimulation (Nirmaljit Kaur, 2000). Bhati and Yadav (2003) suggested that TSS increase in ber (*Zyzyphus mauritiana* Lamk.) fruits was due to increase in mobilization of carbohydrates from source to sink (fruits) by NAA treatments.

Significant increase in soluble sugar (i.e glucose, fructose and sucrose) was found with the application of NAA. The highest soluble sugar content was recorded by foliar application of 100 ppm NAA. The function of auxins which could potentially influence sink strength, in addition the promotion of cell elongation and cell division appears to be regulation of key enzymes involved in sugar metabolism such as acid invertase and enhanced mobilization of assimilates (Morris and Arthur, 1986).

The number of cells larger than 200 µm in the 40-day fruit treated with 4-CPA (40-day CPA fruit) was nearly the same as the number of the cells of the 55 d control fruit. Therefore, it is suggested that the increase in number of large cells caused by 4-CPA treatment of melon fruit at an early stage of fruit development increases the sink strength of cells, which promotes active import of sucrose from an early stage of fruit development (Kano, 2002). Also, Kano (2009) suggested a relationship between cell size and the sugar type accumulation in melon fruit. As small cells have potentialities to further development, the cells suppress sucrose synthesis in the fruit and preferentially accumulate glucose and fructose which are immediately utilized for cell development. On the other hand, sucrose is accumulated preferentially as a reserve substance in larger and fully developed cells because of no necessity to save glucose and fructose for their further development.

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