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Effects of nitrogen treatments and harvesting stages on the aconitic acid, invert sugar and fiber in sweet sorghum cultivars

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Abstract: *Sweet sorghum is adapted to the hot and dry climatic conditions and its tolerance to slat is moderately. It can be used for different products such as food, feed, fiber and fuel. This study was carried out to evaluate the effects, three nitrogen treatments, and three harvesting stages on the aconitic acid, fiber and invert sugar of three sweet sorghum cultivars in the experimental station and the results showed that the effects were significant. Among nitrogen treatments, application of 100 kg ha^{-1} urea at planting and 200 kg ha^{-1} urea at 4 leaf stage had the highest aconitic acid (0.26%) and invert sugar (3.44%). Among sweet sorghum cultivars, IS2325 and Vespa had the highest aconitic acid (0.26%) and invert sugar (3.86%), respectively. Plant harvested at 4 leaf stage had the highest aconitic acid (0.26%) and the highest invert sugar (3.85%). Rio had higher fiber content than Vespa and IS2325 and all cultivars had the highest fiber content before chilling harvesting stage. In general, since high invert sugar and high aconitic acid interfere crystallization of sugar so, it is suggested that to plant Vespa, apply urea 100 kg ha^{-1} urea at planting, 100 kg ha^{-1} urea at 4 leaf stage and 100 kg ha^{-1} urea at booting and harvested before chilling that had lowest aconitic acid and invert sugar. Thereby, it is recommended to plant Vespa, apply urea 100 kg ha^{-1} urea at planting, 100 kg ha^{-1} urea at 4 leaf stage and 100 kg ha^{-1} urea at booting and harvested at 4 leaf stage that had the highest aconitic acid.*

Key words: *Sweet sorghum, Nitrogen treatments, Harvesting stages, Invert sugar, Fiber and aconitic acid*

Introduction

Sweet sorghum [*Sorghum bicolor* L. (Moench)] usually is planted for sugar (Almodares and Sepahi, 1996; Ghaneker *et al.*, 1992) and ethanol production (Gnansounoua *et al.*, 2005). It is often considered as one of the most drought resistant (Tesso *et al.*, 2005) and moderate salt tolerant (Almodares *et al.*, 2007a) agricultural crops. It has the capability of remaining dormant during the dry period. Aconitic acid presents in wheat leaves and may be linked to a hydrophilic molecule such as a sugar (Remus-Borel *et al.*, 2006). It may act as phytoalexins and/or defense molecules (Remus-Borel *et al.*, 2006). Also, it is known as an antifeedant in barnyard grass and rice which may be useful in not feeding the plants by insect (Hattori, 2001). Also, it can be used as a chemotaxonomic marker in some plant families (Loriot *et al.*, 2007). Aconitic acid should not be present in the sugarcane and sweet sorghum juice or the concentration should be low enough not to interfere with crystallization of sucrose (Coleman, 1983). The percentage of juice phosphate is inversely related to the concentration of aconitic acid in the juice. Hence, high levels of aconitic acid increase the buffering capacity of the juice, and cause high mud volumes (Kampen, 2000). The sour syrup taste of sweet sorghum could be due to high aconitic acid level and low invert sugar in the juice (Nimbkar *et al.*, 2006). The effects of nitrogen and harvesting stage on the aconitic acid content in plants are less studied. Nitrogen is essential for plants growth and nitrogen fertilizer is an essential component of increased food (Mosier *et al.*, 2004). Where nitrogen levels are limiting, photosynthesis is not fully used in the synthesis of organic nitrogen compounds and sugars are accumulated (Mengel and Kirkbiy, 1978; Karic *et al.*, 2005). Invert sugar content in *Allium porrum* had no relationship with the level of nitrogen that was applied (Karic *et al.*, 2005; Brunsgard *et al.*, 1997). In contrary, Biczak *et al.* (1998) observed that in celery, increasing pre-sowing and top-dressing doses of nitrogen fertilizers cause a decrease of the sugars in vegetables. After flowering in sweet sorghum, as plant grows sucrose content increased while invert sugar decreased. Total sugar increases through growth stages in sweet sorghum and reach to its highest following stem maturity (Parvatikar and Manjunath, 1991). After flowering in sweet sorghum, sucrose content increases more than glucose and fructose (Bosetto *et al.*, 1986). Sweet sorghum before booting and flowering stages has the minimum amount of sugar content, so there is relationship between sugar content and stages of growth (Vietor *et al.*, 1990). Zanini (1990) in sweet sorghum reported that at the plant maturity invert sugar decreased. Regarding invert sugar, Mathan (1989) reported that at all growth stages of sweet sorghum the amount of glucose is higher than fructose. The yield and concentration of invert sugar in the stalk are highest early in crop growth but decline with crop age (Lingle and Irvine, 1994). This reduction occurs because the number of actively-growing internodes declines and mature internodes lower on the stalk contain little invert sugars (Campbell and Bonnett, 1996). Nitrogen fertilizer increases sugar content in sweet sorghum (Galani *et al.*, 1991). The parcentage

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increased in invert sugars under high nitrogen supply was greatest at the earlier growth stage (Robertson *et al.*, 1996). Nan *et al.* (1994) reported that differences in glucose among sweet sorghum varieties were significant; however there were no significant differences among growth stages, but the stored sample after harvest gave obviously higher glucose content. They indicated that like glucose, fructose increased very significantly after storage. In sweet sorghum, the effects of nitrogen and harvesting stage on the fiber percent are not reported yet. Regarding fiber content in sweet sorghum, it decreases juice extraction that can reduce sugar content. Also it is a critical question in the system analysis as a feedstock or conversion to ethanol and paper production (Worley *et al.*, 1992). However, potential ethanol yield from the fiber is more difficult to predict because emerging enzymatic hydrolysis technology has not been proven on a commercial scale (Rains *et al.*, 1993). The purpose of this study was to evaluate the effects of three nitrogen levels and three harvesting stages on aconitic acid, invert sugar and fiber in three sweet sorghum cultivars.

Materials and Methods

This experiment was carried out at the Isfahan University Research Station, Iran. Three nitrogen treatments; 100 kg ha^{-1} urea at planting, 100 kg ha^{-1} urea at 4 leaf stage and 100 kg ha^{-1} urea at booting (f_1); 100 kg ha^{-1} urea at planting and 200 kg ha^{-1} urea at 4 leaf stage (f_2) and 300 kg ha^{-1} urea at planting (f_3), three sweet sorghum cultivars; Vespa (c_1), Rio (c_2) and IS2325 (c_3) and three harvesting stages; flowering (h_1), physiological maturity (h_2) and before chilling (h_3) were assessed in split-split plot design with three replications. Nitrogen treatments assigned to main plot, cultivars to sub-plots and harvesting stages to sub-sub plot. In May 2003, seeds were planted in furrows 10 m long and 0.5 m apart. Following establishment, plants were thinned to 10 cm apart so that the final populations were 200,000 plants ha^{-1}. Plants were harvested at each harvest stage. Stalks were weighed after the leaves and panicles were removed, then the stalks passed through sugar mill. Aconitic acid and invert sugar of the juice was measured according to Roberts and Ambler (1947) and Lane-Eynon (1970) methods, respectively. Stalk fiber was measured by Varma (1988). Statistical analyses were performed using Statistical Analysis System (SAS) computer program. The means were compared according to Duncan's multiple rang test.

Table - 1: Mean comparisons* among sweet sorghum cultivars for three different levels of nitrogen treatments for characteristic measured

Nitrogen treatments	Aconitic acid (%)	Invert sugar (%)	Fiber (%)
f_1	0.21 b	2.94 b	21.52
f_2	0.26 a	3.44 a	21.67
f_3	0.24 a	2.81 b	21.74

* Values within each column followed by the same letter are not significantly different at $p<0.05$, f_1 = 100 kg ha^{-1} urea at planting, 100 kg ha^{-1} urea at 4 leaf stage and 100 kg ha^{-1} urea at booting, f_2 = 100 kg ha^{-1} urea at planting and 200 kg ha^{-1} urea at 4 leaf stage, f_3 = 300 kg ha^{-1} urea at planting

Table - 2: Mean comparisons* among three sweet sorghum cultivars for aconitic acid, invert sugar and fiber

Cultivars	Aconitic acid (%)	Invert sugar (%)	Fiber (%)
Vespa (C_1)	0.20 c	3.86 a	21.23 b
IS2325 (C_3)	0.26 a	2.78 b	21.14 b
Rio (C_2)	0.25 b	2.55 b	22.56 a

* Values within each column followed by the same letter are not significantly different at $p<0.05$

Table - 3: Mean comparisons* among three sweet sorghum cultivars harvested at flowering, physiological maturity and before chilling stages for characteristic measured

Harvesting stages	Invert sugar (%)	Fiber (%)	Aconitic acid (%)
Flowering (h_1)	3.85 a	11.01 c	0.26 a
Physiological Maturity (h_2)	2.20 c	19.78 b	0.24 b
Before Chilling (h_3)	3.15 b	30.15 a	0.21 c

* Values within each column followed by the same letter are not significantly different at $p<0.05$

Results and Discussion

The results showed the effect of nitrogen treatments on invert sugar and aconitic acid content were significant at 1% level. Table 1 show the amount of invert sugar in f_2 treatment (100 kg ha^{-1} urea at planting and 200 kg ha^{-1} urea at 4 leaf stage) was higher (3.44%) than other nitrogen treatments (2.87%). It seems that translocation of nitrogen in the plant at the four leaf stage is faster then other growth stages. On the other hand, nitrogen assimilation needs carbon which is provided by carbohydrates (Taiz and Zeiger, 2002). Thus, as the amount of nitrogen absorption increased, more carbohydrates are needed. So, the addition of 200 kg ha^{-1} urea at four leaf stage could cause the amount of mono-carbohydrate (invert sugars) increased which may reduce sucrose and other disaccharides or polysaccharide. The amount of aconitic acid in f_1 treatment (100 kg ha^{-1} urea at planting, 100 kg ha^{-1} urea at 4 leaf stage and 100 kg ha^{-1} urea at booting) was lower (0.21%) than other nitrogen treatments (0.25%). Aconitic acid is one of the metabolic compound that is produce in TCA (Taiz and Zeiger, 2002). Therefore, it seems that uniform distribution of nitrogen treatment (f_1) during growing season reduce aconitic acid because it is better consumed in TCA. But, extra addition of nitrogen treatments at the beginning of the season (f_3) or at four leaf stage (f_2) cause aconitic acid to be increased. Preparing good quality syrup needs low invert sugar and aconitic acid. Thus, it is suggested to apply nitrogen uniformly during the growing season (f_1). Table 2 showed the effects of cultivars on the invert sugar, fiber and aconitic acid were significant at 1% level. Aconitic acid content of IS2325 (0.26%) was higher than Vespa (0.20%) and Rio (0.25%). It seems that variations of aconitic acid content in cultivars could be due to their genetics differences because aconitic acid also could be produced in other metabolism pathways. Cv Vespa had the higher invert sugar content

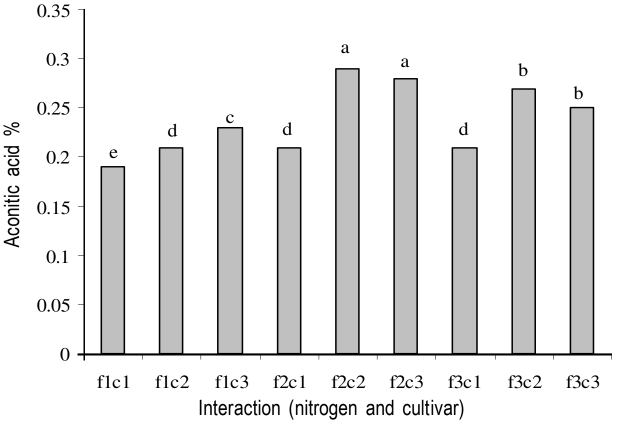

Fig. 1: Interaction between levels of nitrogen treatments and cultivars on aconitic acid; f_1, f_2, f_3 and; c_1, c_2, c_3 are different nitrogen treatments and cultivars respectively

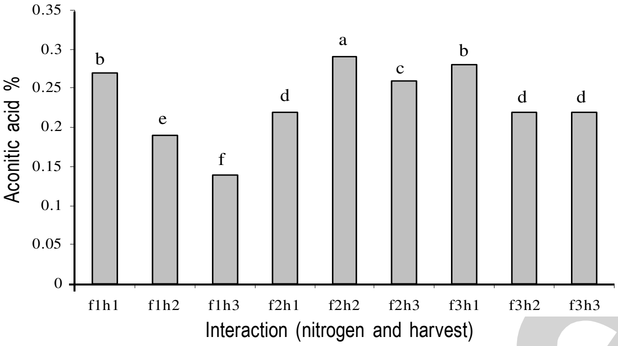

Fig. 2: Interaction between levels of nitrogen treatments and harvesting stages on aconitic acid; f_1, f_2, f_3 and; hr_1, hr_2 and hr_3 are different nitrogen treatments and harvesting stages respectively

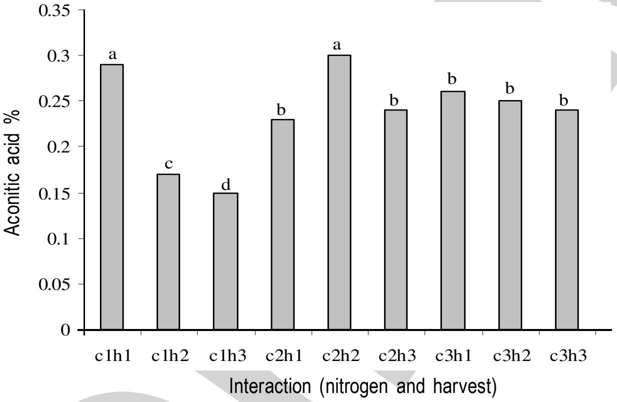

Fig. 3: Interaction between cultivars and harvesting stages on aconitic acid; c_1, c_2, c_3 and; hr_1, hr_2 and hr_3 are different cultivars and harvesting stages respectively

than Rio and IS2325. Almodares *et al.* (2007b) reported that in similar conditions, sucrose content of Vespa was lover than other cultivars. Therefore, it seems that according to Malinovskii and Smilovenko (1988) report may be invert sugar in this cultivar was not used for sucrose synthesis. Cv Rio had higher fiber content than Vespa and IS2325. Rio is a medium maturity cultivars comparing to so its fiber content could be related to its maturity. Thus, it seems that Rio due to its low invert sugar and aconitic acid content is more suitable than Vespa and IS2325 for crystal sugar making. Table 3 showed the effects of harvesting stages on invert sugar, fiber and aconitic acid content were significant at 1% level. The amount of aconitic acid was highest and lowest at flowering (0.26%) and before chilling (0.21%), respectively. Ghaneker *et al.* (1992) reported that in sorghum as plant grows the amount of its aconitic acid decreases through maturation. This reduction could be due to disintegration of acid or combination with other substances. Also, Kampen (2000) indicated that high aconitic acid levels typify juice from areas where cane is harvested while still slightly immature which is agreement with our results in sweet sorghum. Invert sugar content was highest at flowering (3.85%) and lowest at physiological maturity (2.20%). Zanini (1990) reported that in sweet sorghum at flowering, the amount of invert sugar was higher than physiological maturity because at flowering the amount of sucrose which converts to invert sugar was lower than physiological maturity. His findings were in agreement with our results. On the other hand, Nan *et al.* (1994) indicated that in sweet sorghum, harvesting stages had no significant differences in sugar content, meaning that sugar content was stable during that period. It may be because their sampling intervals were short. Fiber content was highest before chilling stage (30.15%) and lowest at flowering stage (11.01%). Zanini (1990) reported that structural carbohydrate such as fiber increased as plant matures which is in agreement with our results. Therefore, it seems that low aconitic acid, invert sugar and fiber could be obtained when sweet sorghum is harvested at physiological maturity which is suitable for producing high quality juice in crystallization of sugar. The interaction between nitrogen treatments and cultivars for aconitic acid content was significant at 1% level. In treatment f_2, IS2325 and Rio had the highest aconitic acid (0.29%). Also, Vespa had the lowest aconitic acid content (0.19%) in f_1 treatment (Fig. 1). All the cultivars had the minimum aconitic acid in f_1 treatment while f_2 treatment increased aconitic acid. In this relation, large amount nitrogen fertilizer reduces plant carbohydrate but if dose not absorb by the plant, its carbohydrate content increase. Thus, time of fertilizer application should be in such a time not to reduce carbohydrate content at plant maturity. When nitrogen absorbs by the plant it incorporated in amino acid molecule so, some of the metabolites in TCA cycle is used and the continuation of the cycle needs supply of the carbohydrates. Therefore addition of nitrogen reduces carbohydrate content in the plant. Thereby, it seems that application of 200 kg ha^{-1} urea at 4 leaf stage (f_2) which plant is growing, caused the reduction of carbohydrates in TCA so accumulation of aconitic acid could be due to the secondary pathway rather than TCA. The secondary metabolism pathway such as aconitic acid could be produce under stress conditions (Palumbo *et al.*, 2007) and uneven distribution of nitrogen application may be one of this stress conditions. High amount of nitrogen application at planting (f_3) causes the reduction of aconitic acid. It seems that the extra nitrogen may be leached out from the soil because the growth of the plant is not sufficient to absorb nitrogen at planting stage. On the other hand, as Walford (1998) reported that the amount of aconitic acid in cane growing is low because it is used in the Krebs cycle and not stored in the plant. Therefore, it seems that in f_1 and f_3 treatments, carbohydrates were used in Krebs cycle and aconitic acid was not stored in the plant. The interaction between nitrogen treatments and harvesting stage for aconitic acid content was significant at 1% level. The highest amount of the aconitic acid (0.29%) was obtained at physiological maturity when urea applied at f_2 treatment (Fig. 2). Also, the lowest amount of aconitic acid (0.14%) was observed at before chilling when urea used at f_1 treatment. Harvesting before chilling stage had the lower aconitic acid content than other two harvesting stages across all nitrogen treatments (Fig. 2). Furthermore, as harvesting stage delayed the amount of aconitic acid decreased significantly at f_1 treatment. Our results are in agreement with Kampen (2000) reports who indicated that high aconitic acid levels typify juice from areas where cane is harvested while still slightly immature. Therefore, it seems that as the plant matured the amount of aconitic acid decreased that may be converted to other chemicals. The interaction between cultivars and harvesting stages on aconitic acid was significant 1% level. Vespa and IS2325 had the highest aconitic acid content at 4 leaf stage and physiological maturity (0.29, 0.30%), respectively which was not significantly deferent. Vespa had the lowest aconitic acid before chilling stage and as harvesting stage delayed the amount of aconitic acid decreased significantly. In conclusion, since invert sugar and aconitic acid interfere crystallization of sugar, so the cultivars, nitrogen treatments and harvesting stages should be used that have the lowest invert sugar an aconitic acid. It is suggested that to plant Vespa and apply 100 kg ha^{-1} urea at each stage of planting, 4 leaf stage and booting; and harvested before chilling.

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