



## Effects of nitrogen fertilizer and plant growth regulator on stalk yield and bioethanol in sweet sorghum

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### Abstract

In this study, the effects of nitrogen fertilizer and plant growth regulator (ethephon) application on leaf chlorophyll, stem height and diameter, stalk yield, total sugar and bioethanol of sweet sorghum were determined. Four nitrogen treatments 0, 100, 200 and 300 Kg Urea ha<sup>-1</sup> and four ethephon concentrations 0, 800, 1000, and 1200 ppm were applied on sweet sorghum in a split plot on randomized complete block design with three replications. The results showed that interaction of nitrogen and ethephon on stalk yield was significant. The highest stalk yield (30.91 t ha<sup>-1</sup>) was obtained with the application of N<sub>4</sub>E<sub>4</sub> (300 kg Urea ha<sup>-1</sup> and 1200 p.p.m (ethephon) and the lowest (12.15 t ha<sup>-1</sup>) with control and N<sub>2</sub>E<sub>1</sub> (100 kg Urea ha<sup>-1</sup> and 0 ppm ethephon). Maximum amount of total sugar (12.06 %) was obtained at 300 Kg Urea ha<sup>-1</sup>. The lowest bioethanol was 2423 L ha<sup>-1</sup> at 0 ppm ethephon and the highest was 4424 L ha<sup>-1</sup> at 1200 ppm ethephon. Based on these results, it is suggested to apply 300 Kg Urea/ha and ethephon at 1200 ppm to have the highest bioethanol in sweet sorghum.

**Keywords:** nitrogen; ethephon; sweet sorghum; biomass; bioethanol

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### Introduction

Sorghum (*Sorghum bicolor* L. Moench) is well adapted to sub-tropical and temperate regions of the world and is used in different ways in many countries. Sweet sorghum is consumed as food and feed (Almodares et al., 2007) and used for sugar, ethanol and paper pulp production (Gnansounous et al., 2005). The

essence of sweet sorghum is from its stalk, which contains high sugar content (Almodares et al., 2008). In addition, sweet sorghum is a C4 crop with high photosynthetic efficiency. Crop management is important to attain higher stalk yield in sweet sorghum. Among the various inputs that improve the efficiency of a cultivar in realizing its potential, fertilizers (nitrogen in particular) play a crucial role. Nitrogen is generally a limiting nutrient in sorghum production. To achieve economically viable

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returns, efficient use of available resources, like nitrogen, is necessary to maximize yields in all seasons. Variable responses to the application of nitrogen fertilizer have been observed in sorghum owing to differences in climatic, soil and genotypic factors across seasons and locations (Muchow, 1998). Application of nitrogen fertilizer increased sweet sorghum stalk yield (Johnston, 2000). Mengel and Kirkby (2001) mentioned that corn and sorghum yield would have dropped by 41% and 19%, respectively, without nitrogen fertilizer application. Ethephon (2-chloroethylphosphonic acid), is an ethylene-generating compound that has a profound effect on plant growth and developmental processes. The response of ethephon depends on the species, cultivar, rate and time of application (Foster et al., 1991). These same researchers also reported that ethephon significantly increased biomass and tillering of barley while the plant height was reduced. The inhibition of flowering in sugarcane by ethephon was reported by Moore and Osgood (1989). They also demonstrated 87% reduction in tasseling, 7.5% increase in yield, and 10% increase in sugar yield. Sorghum has been indicated as one of the difficult plant species for genetic manipulation (Pola et al., 2008). As a result, using other methods should be considered to increase carbohydrates and biomass. Therefore, it is of considerable value to carry out an experiment on biomass and bioethanol of sweet sorghum in relation to different rates of nitrogen fertilizer and ethephon.

## Materials and Methods

Field experiment was conducted at the

University of Isfahan Experimental Station in 2010. Four nitrogen treatments including 0, 100, 200 and 300 Kg Urea ha<sup>-1</sup>, and four ethephon concentrations including 0, 800, 1000 and 1200 ppm were assessed in a split plot randomized complete block design with three replications. Nitrogen and ethephon treatments were assigned to main plot and sub-plot, respectively. The plots received 150 kg/ha of superphosphate triple and 100 kg/ha of potassium sulphate. Then the soil was mixed with these fertilizers before planting. In May 2010, seeds were planted in furrows 4 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. Nitrogen fertilizer was applied in 3 stages: pre-sowing, 8 leaf and flowering stage. Ethephon with the mentioned concentrations was sprayed at booting stage. At flowering stage, leaf chlorophyll was measured with chlorophyll meter (model SPAD – 502). At every plot three meters from two center rows were harvested when the plants reached at physiological maturity. After harvesting, growth parameters including plant height and plant diameter were measured. Stalks were weighed after the leaves and panicles were removed. The stalks were passed through sugar mill and total sugar and bioethanol were measured according to Wang and Liu (2009) and Lipinsky et al. (1978) method, respectively.

## Results

Table 1 shows analysis of variance for nitrogen treatments and ethephon concentrations on leaf chlorophyll, stem height

Table 1

Analysis of variance of leaf chlorophyll, stem height and diameter, stalk yield, total sugar and bioethanol at different nitrogen treatments and ethephon concentrations of sweet sorghum

Source	df	Leaf chlorophyll	Stem height	Stem diameter	Stalk yield	Total sugar	bioethanol
Block	2	<sup>ns</sup> 54.12	<sup>ns</sup> 52.260	<sup>ns</sup> 09.8	<sup>ns</sup> 93.25	<sup>**</sup> 90.18	<sup>ns</sup> 318.305011
Nitrogen (A)	3	<sup>*</sup> 37.15	<sup>*</sup> 26.1114	<sup>*</sup> 82.10	<sup>**</sup> 58.109	<sup>**</sup> 92.4	<sup>**</sup> 979.5308369
Error a	6	02.3	89.232	67.1	99.8	37.0	461.147970
Ethephon (B)	3	<sup>**</sup> 1647.30	<sup>ns</sup> 84.520	<sup>*</sup> 37.6	<sup>**</sup> 17.205	<sup>**</sup> 52.9	<sup>**</sup> 435.8834826
A*B	9	<sup>ns</sup> 40.2	<sup>ns</sup> 05.429	<sup>ns</sup> 65.2	<sup>*</sup> 99.14	<sup>ns</sup> 58.0	<sup>ns</sup> 920.420726
Error b	24	60.2	61.228	45.1	22.5	70.0	733.205824
Total	47						

<sup>\*\*</sup>, <sup>\*</sup>, <sup>ns</sup> Significant at 1% level, 5% level and non significant respectively

and diameter, stalk yield, total sugar, and bioethanol. The results showed that the effect of nitrogen fertilizer was significant at 1% level on stalk yield, total sugar, and bioethanol and at 5% on chlorophyll, stem height and diameter. The mean comparison of the nitrogen fertilizer levels on the above measurements is presented in Table 2. Results showed that leaf chlorophyll was increased significantly by increasing nitrogen fertilizer levels. Leaf chlorophyll content was highest under nitrogen level 300 kg Urea ha<sup>-1</sup>, although there was no significant difference with nitrogen level 200 kg Urea ha<sup>-1</sup>. Stem height was increased by increasing nitrogen fertilizer levels. While the highest stem height (186.5 cm) was observed in 300 kg Urea ha<sup>-1</sup>, there was no significant difference in this parameter under 100, 200 and 300 kg Urea ha<sup>-1</sup> treatments. Similar to stem height, the highest stem diameter was 12.65 mm in 300 kg Urea ha<sup>-1</sup> and its lowest was 10.48 mm in 0 kg Urea ha<sup>-1</sup>. Stalk yield was increased significantly by increasing nitrogen fertilizer levels. The lowest (16.12 t ha<sup>-1</sup>) was observed in control and the highest (23.31 t ha<sup>-1</sup>) at 300 kg Urea ha<sup>-1</sup>. Sweet sorghum had the highest total sugar (12.06%) at 300 kg Urea ha<sup>-1</sup>. Mean comparison also indicated that bioethanol increased significantly with increasing nitrogen levels. It was highest (4243 l ha<sup>-1</sup>) at 300 kg Urea ha<sup>-1</sup>.

Results of mean comparison for the effects of ethephon concentrations on the

measured traits are presented in Table 3. Mean comparison of ethephon concentration on leaf chlorophyll showed that the highest concentration (48.47 mg cm<sup>2</sup>) was obtained in 800 ppm ethephon and it was a significantly different from other ethephon concentrations. Stalk yield was significantly different at all ethephon concentrations. The lowest (14.93 t ha<sup>-1</sup>) and highest (24.51 t ha<sup>-1</sup>) yields were obtained at control and 1200 ppm ethephon, respectively. Total sugar was significantly affected (P<0.05) by ethephon. It was increased by increasing ethephon concentrations from 0 to 1200 ppm, although the concentrations 800, 1000 and 1200 ppm were not significantly different. Also the highest bioethanol (4424 l ha<sup>-1</sup>) was obtained with application of 1200 ppm ethephon. The effect of interaction between nitrogen and ethephon was significant (P<0.05) only on stalk yield (Fig. I).

## Discussion

Buah and Mwinkaara, (2009) showed that leaf chlorophyll content in sorghum was increased by increasing nitrogen fertilizer levels. This is in agreement with our findings. Nitrogen is one of the major components of chlorophyll. Thus, it seems that with increasing soil nitrogen levels, nitrogen availability in leaf and consequently leaf chlorophyll content increase. In

Table 2

Mean comparison\* of leaf chlorophyll, stem height and diameter, stalk yield, total sugar and bioethanol at nitrogen treatments

Nitrogen Fertilizer (Kg/ha)	Leaf chlorophyll (mg cm <sup>2</sup> )	Stem height (cm)	Stem diameter (mm)	Stalk yield (t ha <sup>-1</sup> )	Total Sugar (%)	Bioethanol (L ha <sup>-1</sup> )
0	24 <sup>b</sup> .45	166b	48b.10	12c.16	61c.10	2675d
100	10 <sup>ab</sup> .46	2ab.175	35ab.8	34bc.18	15bc.11	3213c
200	47 <sup>a</sup> .47	4a.185	15a.12	92b.19	75ab.11	3655b
300	59 <sup>a</sup> .47	5a.186	65a.12	31a.23	06a.12	4243a

\* Values within a column followed by the same letter are not significantly different at P<0.05 using Duncan multiple range test.

Table 3

Mean comparison\* of leaf chlorophyll, stem diameter, stalk yield, total sugar and bioethanol at ethephon concentrations

Ethephon concentration (p.p.m)	Leaf chlorophyll (mg cm <sup>2</sup> )	Stem diameter (mm)	Stalk yield (t ha <sup>-1</sup> )	Total Sugar (%)	Bioethanol (L ha <sup>-1</sup> )
0	63c.44	77b.10	93d.14	09b.10	2423d
800	47a.48	48a.12	55c.17	55a.11	3149c
1000	31b.46	93a.11	70b.20	97a.11	3790b
1200	47b	11.45ab	24.51a	96a.11	4424a

\* Values within a column followed by the same letter are not significantly different at P<0.05 using Duncan multiple range test.

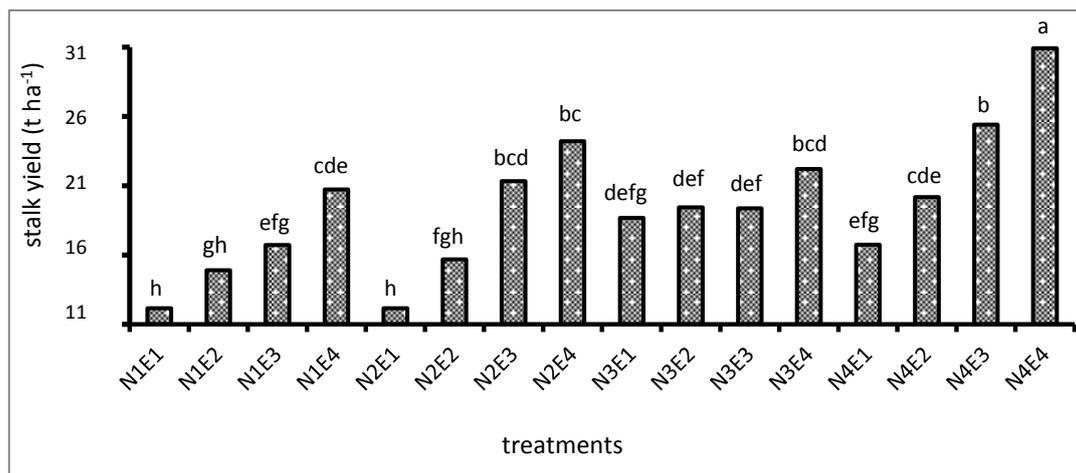


Fig. 1. Interaction between nitrogen and ethephon on stalk yield; Values by the same letter are not significantly different at  $P < 0.05$  using Duncan multiple range test.  $N_1 = 0$ ,  $N_2 = 100$ ,  $N_3 = 200$ ,  $N_4 = 300$  Kg/ha(Urea),  $E_1 = 0$ ,  $E_2 = 800$ ,  $E_3 = 1000$ ,  $E_4 = 1200$  ppm

other words, nitrogen application causes more photosynthetic area and leaves active growth and increases assimilate absorption and transmission due to leaf area improvement. The results for stem height and diameter are quite in line with those of Almodares et al. (2008) who showed that nitrogen fertilizer increased stem height and diameter. Nitrogen may affect plant growth through cell division and cell enlargement which consequently increase stem height and diameter (Stals and Inze, 2001). In our experiment ethephon had no significant effect on stem height. In contrast, Foster et al. (1991) tested ethephon at 0.3 and 0.6 kg ha<sup>-1</sup> on barely (*Hordeum gate* L.) and reported that plant height was reduced by ethephon application. Also the other researchers reported that ethephon application to bluegrass (*Poa pratensis* L. var Sydspport) at 10,000 ppm increased stem length more than twenty-fold (Poovaiah and Leopold, 1973). The lack of responsiveness of sweet sorghum plant height to ethephon application could be due to low ethephon concentration in our experiment active ingredient (a. i.) 30%. Results of stalk yield are similar to those reported by Johnston (2000). Nitrogen is a major element essential for synthesis of amino acids, nucleic acids and some organic acids. It is necessary for plant growth and development and its shortage reduces yield (Zaho et al., 2005). Humm (2001) reported that ethrel (ethephon 48%) applied to sugarcane crops at the rate of 1.0-1.5 l ha<sup>-1</sup> was highly effective in suppressing

flowering. This had beneficial effects on the yield of crops harvested. Using ethrel to suppress flowering is suggested as a standard practice for such crops, if weather is conducive to flower initiation early in the year. Also, in evaluating the effect of ethephon on herbaceous perennials the results showed that spraying ethephon three times at 1000 mg l<sup>-1</sup> on *Echinacea purpurea*, *Monarda didyma* and *Physostegia virginiana* Bentham delayed flowering 6, 7 and 9 days, respectively (Hayashi et al., 2001). So ethephon is useful for prolong vegetative growth period and more stalk yield. Therefore ethephon application is advisable for bioethanol production from sweet sorghum. Wei et al, 2006 reported that ethephon causes photosynthesis improvement; therefore, our results can be due to this reason. Li and Solmon, (2003) reported that biomass productivity per unit area increased in sugarcane following ethephon treatment. Sugar yield also increased with increase in the rates of nitrogen (Reddy et al., 2008). Tallat (2002) reported that nitrogen fertilization had site-specific effects on quantitative traits (sugar yield). Combined data over years and at different sites suggests that fresh roots and sugar yields were maximized at high nitrogen rates (Ayub et al., 2002). These are in line with our results. Moore and Osgood (1989) showed 87% reduction in tasseling in the ethephon treated blocks. The yield of sugarcane was increased by 7.5% and the yield of sugar by 10%. In another experiment on the effect of plant growth regulators (Sulfometoron metal and

ethephon) on sugarcane, it was found that regulators controlled the flowering and increased the sugar production (Caputo et al., 2007). Liao et al. (2003) reported that ethephon increased sugar content per fruit more rapidly than control. They explained that such results may be due to ethylene effect on sugar accumulation in fruit tissues. This is in agreement with our findings. Management in terms of nitrogen inputs and also plant growth regulators is critical to increase the stalk yield. Coutinho et al. (1998) observed that cane and ethanol yields increased with application of up to 100 kg Urea ha<sup>-1</sup>.

High fermentable sugar content in sweet sorghum stalk makes it more suitable for fermentation to ethanol. As sweet sorghum has high amount of sucrose, glucose and fructose which is easily converted to ethanol by microorganisms, it is a suitable plant for biofuel production. Sweet sorghum juice is assumed to be converted to ethanol at 85% theoretical, or 54.4 L ethanol per 100 kg fresh stalk yield (Almodares and Hadi, 2009). Improving the operational scheming of breeding approach to quantitatively increase the juice yield and the fermentation efficiency through increased sugar content can help meet the energy requirements of future generations. Based on the results of the experiment, it can be concluded that for obtaining maximum sugar yield and bioethanol in sweet sorghum, 300 kg Urea ha<sup>-1</sup> and 1200 ppm ethephon can be applied.

## References

- Almodares, A., R. Taheri and S. Adeli.** 2007. Inter-relationship between growth analysis and carbohydrate. *Journal Environmental Biology*, 28 (3): 527-531.
- Almodares, A., R. Taheri, I. M. Chung and M. Fathi.** 2008. The effect of nitrogen and potassium fertilizers on growth parameters and carbohydrate contents of sweet sorghum cultivars. *Journal Environmental Biology*, 29: 849-852.
- Almodares, A and M. Hadi.** 2009. Production of bioethanol from sweet sorghum: A review. *African Journal of Agricultural Research*, 4 (9): 772-780.
- Ayub, M., M. A. Nadeem, A. Tanveer and A. Husnain.** 2002. Effect of different levels of nitrogen and harvesting times on the growth, yield and quality of sorghum fodder. *Asian Journal of Plant Science*, 1(4): 304-307.
- Buah, S. S. J and S. Mwinkaara.** 2009. Response of sorghum to nitrogen fertilizer and plant density in the guinea savanna zone. *Agronomy Journal*, 8(4): 124-130.
- Caputo, M. M., M. A. Silva, E. G. F. Beauclair and G. J. C. Gava.** 2007. Sugarcane sucrose accumulation, productivity and flowering using plant regulators. *Interciencia*, 32: 834-840. (in Portuguese with English summary)
- Coutinho, E. L. M., J. C. Fares, A. G. Pinto and E. C. A. Souza.** 1988. Effect of nitrogen and potassium fertilizer on sweet sorghum (*Sorghum bicolor* L. Moench) evaluated by foliar diagnosis and culm and ethanol production. *Cientifica (Brazil)*, 16: 261-270.
- Foster, K., D. Reid and J. Taylor.** 1991. Tillering and yield responses to ethephon in three barley cultivars. *Crop Science*, 31 (1): 130-134.
- Gnansounou, E., A. Dauriat and C. E. Wyman.** 2005. Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. *Bioresource. Technology*, 96(9): 985-1002.
- Hayashi, T., R. D. Heins, A. C. Cameron and W. H. Carlson.** 2001. Ethephon influences flowering, height, and branching of several herbaceous perennials. *Science horticulture*, 91: 305-324.
- Humm, M.** 2001. Observations of the suppression of sugarcane flowering using ethephon on the KwaZulu-Natal South Coast. *African Sugar Technology*, 75: 187-191.
- Johnston, A. E.** 2000. Efficient use of nutrients in agricultural production systems. *Communications in soil science and plant analysis*, 31(11-14): 1599-1620.
- Li, Y. and S. Solomon.** 2003. Ethephon: a versatile growth regulator for sugar cane industry. *Sugar Technology*, 5 (4): 213-223.
- Liao, W. Z., Y. R. Li, Y. K. Lin, Y. Y. Nong, Y. Liu and L. T. Yang.** 2003. Effects of ethephon applied at different times of late growth stage on ripening of sugarcane. Southwest

- China. *Journal of Agricultural Sciences*, 16 (4): 60-64.
- Lipinsky, E. S., H. S. Birkett, J. A. Polack, J. E. Atchison, S. Kresovich, T. A. McClure and W.T. Lawhon.** 1978. Sugar crops as a source of fuels. *Volume 2. Processing and conversion research*.
- Moore, P and R. Osgood.** 1989. Prevention of flowering and increasing sugar yield of sugarcane by application of ethephon (2-chloroethylphosphonic acid). *Journal of Plant Growth Regulator*, 8 (3): 205-210.
- Mengel, K and E. A. Kirkby.** 2001. *Principles of plant nutrients. 5th ed.* Kluwer Academic Publishers.
- Muchow, R. C.** 1998. Nitrogen utilization efficiency in maize and grain sorghum. *Field crops research*, 56(1-2): 209-216.
- Pola, S., N. S. Mani and T. Ramana.** 2008. Plant tissue culture studies in Sorghum bicolor: immature embryo explants as the source material. *International Journal of Plant Production*, 2 (1): 1-14.
- Poovaiah, B. W and A. C. Leopold.** 1973. Effects of Ethephon on Growth of Grasses. *Crop Science*, 13(6): 755-758.
- Reddy, P. S., B. V. S. Reddy, A. A. Kumar and P. S. Rao.** 2008. Standardization of nitrogen fertilizer rate for sugar yield optimization in sweet sorghum. *Journal of SAT Agricultural Research*, 6: 1-4.
- Stals, H and D. Inzé.** 2001. When plant cells decide to divide. *Trends in Plant Science*, 6(8): 359-364.
- Tallat, M.K.** 2002. Effect of different levels and sources of potassium on growth, yield, and quality of Sugarcane. *Better Crop International*, 16 (1): 14-15.
- Wang, F and C. Z. Liu.** 2009. Development of an Economic Refining Strategy of Sweet Sorghum in the Inner Mongolia Region of China. *Energy and Fuels*, 23: 4137-4142.
- Zhao, D., K. R. Reddy, V. G. Kakani and V. R. Reddy.** 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *European Journal of Agronomy*, 22: 391-403.