

# The influence of increased plant densities and nitrogen fertilizer levels on forage yield, seed yield and qualitative characteristics of forage sorghum (*Sorghum bicolor* L. Moench)

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## ABSTRACT

Nitrogen fertilization is a major agronomic practice that affects the yield and quality of crop. A combined split-plot factorial design based on randomized completely block design (RCBD) with four replications was used to determine the influence of plant density and nitrogen fertilizer on forage and seed yield of sorghum. The main plots were years, namely 2009 and 2010. The subplots were 250,000, 300,000, 350,000 and 400,000 plants/ha, and sub-subplots were four levels of nitrogen fertilizers, namely 0, 80, 160 and 240 kg N/ha. The results obtained from statistical analysis showed that the higher stem diameter and dry forage yield were obtained for 2010 than 2009. The maximum plant height, number of leaves per plant, leaf area index (LAI), fresh forage yield and dry forage yield was achieved in 400,000 plants/ha. At 250,000 plants/ha, the highest number of seeds per panicle (2,484), a thousand seed weight (7.72 g) and seed yield (5.58 t/ha) were obtained. There is no difference in prussic acid among treatments. The highest protein and ash percentages of the first cut were related to 350,000 (11.19%) and 250,000 plants/ha (12.16%), respectively. There were not any significant differences in terms of plant height, number of tillers per plant, stem diameter, prussic acid and ash percentage between the levels of nitrogen fertilizers. The highest fresh forage yield and dry forage yield was achieved in 240 kg N/ha and 160 kg N/ha, respectively. An adequate supply of N to crops is fundamental to optimize crop yields and crop production. The higher a thousand seed weight and seed yield obtained for 160 kg N/ha than those of other treatments, which were 8.04 g and 7.18 t/ha. Grain yield is probably the most important characteristic on which to base nitrogen fertilizer application and selection of appropriate plant density. Moreover, the application rate of 160 kg N/ha had obtained the highest amount of protein percentage of the first cut (11.17%). With appropriate usage of nitrogen fertilizer and improving grain yield accompanied with qualitative traits, sustainability in sorghum production will appear.

**Keywords:** Plant population, fertilizer, protein percentage, ash percentage

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## INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench), the fifth most important cereal crop in the world, is one of the most important staple crops in Africa and South Asia and represents the only viable food grain for many of the world's most food insecure people (Xin *et al.*, 2009; Shahrajabian *et al.*, 2011; Soleymani *et al.*, 2011; Jampala *et al.*, 2012; Soleymani *et al.*, 2012a; 2012b; Zou *et al.*, 2012; Abugri *et al.*, 2013). Forage production is one of the most important agricultural activity, and the key to the successful development of an efficient forage production strategy involves agronomical managements (Esfandiary *et al.*, 2012; Soleymani and Shahrajabian, 2012a; 2012b; Shahrajabian *et al.*, 2013; Soleymani and Shahrajabian, 2013; Soleymani *et al.*, 2013; Soleymani *et al.*, 2016; Yong *et al.*, 2017; Yong *et al.*, 2018). Sorghum is becoming an increasingly important forage crop in many arid and semi-arid regions of the world because of its advantage over other forage crops (Carmi *et al.*, 2006; Rostamza *et al.*, 2011; Jahanzad *et al.*, 2013).

Forage sorghum is an important silage crop for beef and dairy producers. This crop is well adapted to environments with limited rainfall, high temperatures and low soil fertility (Rooney, 2004; Patil, 2013). Plant density can affect forage yield (Cusicanqui and Lauer, 1999) and quality (Defoor *et al.*, 2001). Khalili Moheleh *et al.* (2007) concluded that the maximum protein and ash percentages were obtained for 250,000 plants/ha (11.27%) and 300,000 plants/ha (12.37%), respectively. Soleymani *et al.* (2012a) concluded that the highest seed yield, biological yield and appropriate one hundred seed weight of sweet sorghum was related to 600,000 plants/ha. Soleymani *et al.* (2011) showed that plantation of grain sorghum with 100,000 plants/ha and 200 kg N/ha was appropriate for the climatic condition of Isfahan. Performance of the crop can be improved through manipulation of plant population (Tabo *et al.*, 2002). A decrease in dry matter yield and an increase in forage quality with

reduced plant population in forage sorghum occurred (Caravetta *et al.*, 1990). Cho *et al.* (2004) indicated that crude protein content influenced by plant density, and ash content changed from 8.4% to 6.8% as plant density was decreased. Nitrogen ranks first among the applied inputs to maximize output in agriculture and its demand increasing at a rate approximately equivalent to the rate of increase in population, of about 2% per annum (Zhao *et al.*, 2003; Zhao *et al.*, 2005; Broumand *et al.*, 2010; Jothamani, 2012; Soleymani and Shahrajabian 2012a; 2012b). Beyaert and Roy (2005) reported that maximum yields of forage sorghum were obtained at 125 kg N/ha. Showemimo *et al.* (2002) noticed that highly significant differences were obtained among nitrogen levels for dry matter weight. The experiment of Beyaert and Roy (2005) on forage sorghum–sudangrass in southern Ontario concluded that optimum yield and N efficiency occurred when 100 kg N/ha was applied as a split application. Kaizzi *et al.* (2012) also indicated that above-ground biomass and grain yield of sorghum were significantly affected by nitrogen fertilizer. Javanmard *et al.* (2006) showed that the highest grain yield was obtained in 300 thousand plants/ha, but it was not significantly different from 250 thousand plants/ha. Kazemi-Arbat *et al.* (2000) stated that applying 200 kg N/ha produced the highest dry and fresh-feed yield, but it was not significantly different from 150 kg N/ha. Glamoclija *et al.* (2011) showed that a suitable nitrogen dose of 80–120 kg was the best for three cultivars of sudangrass and fodder sorghum. Millner *et al.* (2011) reported that crude protein for different cultivars of sorghum and sudangrass ranged from 10.3–18.0%. Prussic acid is not normally present in plants, however, under certain conditions, several species of plants can accumulate large quantities of cyanogenic glycosides, which are converted to prussic acid. So, the aim of this study is to determine the influence of plant densities and nitrogen fertilizer on seed yield, protein yield, ash yield, prussic acid, yield components and forage quality of sorghum.

## MATERIALS AND METHODS

### Experimental Site

The study was carried out in 2009 and 2010 at Research Farm, Faculty of Agriculture, Islamic Azad University, Khorasgan Branch, Isfahan, Iran (Latitude 32° 40' N, longitude 51° 58' E and 1,570 m elevation).

### Treatments and Experiment Layout

A combined split-plot factorial design based on randomized completely block design (RCBD) with four replications was used to determine the influence of plant density and nitrogen on forage and seed yield of sorghum. The main plots were years, namely 2009 and 2010. The subplots were 250,000, 300,000, 350,000 and 400,000 plants/ha, and sub-subplots were four levels of nitrogen fertilizer, namely 0, 80, 160 and 240 kg N/ha. In 2009, the field was under cultivation of barley during the previous winter and planting of sorghum was done just after harvesting of barley. In this experiment, a hybrid of forage sorghum, namely, Speed Feed was used. Speed Feed is characterized by early flowering, early maturation, rapid and high accumulation of dry matter and high resistance to weeds and insects. The field was tilled to a depth of 20 cm. The previous crop was harvested on 21 June in 2009 and forage sorghum seeds were sown on 24 June with skillful workers in both 2009 and 2010.

Application of nitrogen fertilizer for each treatment was done in two stages (half of it was used before seed plantation and half of it was used before stem elongation). The source of nitrogen fertilizer was urea. According to soil analysis and a high amount of P and K in the soil, P and K were not used. For sorghum, atrazine [6-chloro-N-Ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] at 1.5 kg a.i./ha and metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] at 1.3 kg a.i./ha were applied within a day after planting to control post-emergence of weeds. A week after germination, the experimental field was retreated against weeds, by using

2,4-D. At the 3–4 leaf stage, the seedlings were thinned with expected densities. Also, weeds were controlled by hoe weeding. The first irrigation was applied immediately after sowing. The second irrigation was done three days after the first one. The other irrigation intervals were done according to the plant's requirement (10 days).

### Data Collection

Each plot had six rows, the length and width of each row was 4 and 3 m, respectively. The distance between rows was 50 cm. Row numbers 1, 4 and 6 and up to 50 cm, primer and edge lines were discarded from sampling. Line numbers 2 and 3 were used for final sampling. Five seeds per hill were sown and later thinned down to two seedlings per stand two weeks after sowing. For assessment of forage parameters in one cutting, 10 samples were harvested when plants were in 20% of anthesis stage. For investigation grain yield and other parameters' samples were taken when plants were in dough stage seed (12–14%). In each subplot, stem height, number of leaves and number of tillers on the main stem were taken in the field. After determination of fresh forage yield, plants were divided into separate organs and dried at 60°C for 96 h in an aerated oven. Dry matter yield of crops considered for comparison of yield production of the treatments. Grain sorghum yield was quantified at physiological maturity and was adjusted to 14% moisture. For evaluating forage parameters in one cutting, 10 samples were harvested. After determination of fresh forage yield, plants were divided into separate organics and dried at 60°C for 96 h in an aerated oven. Ash content was determined by incinerating the samples in a muffle furnace at 550°C for 4 h (Soleymani and Shahrajabian, 2012a). The amount of nitrogen was calculated by Kjeldahl analysis from dry samples and then multiplied nitrogen by 6.25 to determine protein content (Soleymani and Shahrajabian, 2012b). Prussic acid was measured by titration procedure. The titration procedure using silver nitrate with p-dimethylamino-benzal-rhodanine indicator is used

for measuring concentrations of cyanide exceeding 1 mg/L (0.25 mg/250 mL of absorbing liquid).

### Soil Analysis

A soil test for available nitrogen in the soil profile is encouraged where nitrogen application has been excessive relative to yields. The profile nitrogen soil test is used to reduce nitrogen application so accumulated available nitrogen fertilizer is utilized. Another important consideration

in determining the optimum nitrogen rate is the cropping sequence. In this experiment, soil analysis was done at two depths, 0–30 and 30–60 cm in 2009 after the harvest of barley. Electrical conductivity (EC) at two depths of 0–30 and 30–60 cm were 1.9 and 1.6 dS/m, respectively. Furthermore, organic carbon was 0.94 and 1.01% for 0–30 and 30–60 cm, respectively. The soil texture for both depths was Si–Cl (Table 1).

**Table 1** Soil analysis of agriculture research field in Esfahan at two depths (0–30 and 30–60 cm)

| Depth (cm) | SP | EC (dS/m) | pH   | TNV | OC (%) | N (%) | P (ppm available) | K (ppm available) | Gypsum (%) | S (%) | Si (%) | C (%) | Soil texture |
|------------|----|-----------|------|-----|--------|-------|-------------------|-------------------|------------|-------|--------|-------|--------------|
| 0–30       | 60 | 1.9       | 7.95 | 38  | 0.94   | 0.09  | 27.7              | 359               | 0.0        | 17    | 44     | 39    | Si.Cl        |
| 30–60      | 61 | 1.6       | 8.00 | 38  | 1.01   | 0.10  | 32.5              | 361               | 0.0        | 20    | 41     | 39    | Si.Cl        |

**Note:** SP = saturation percentage, EC = electrical conductivity, TNV = total neutralizing value, OC = organic carbon

### Water Analysis

Water analysis from the water sample of the agriculture research field is shown in Table 2.

The water salinity was suitable for the plantation of sorghum. The sodium adsorption ratio (SAR) and pH of water analysis were 6.2 and 7.65, respectively.

**Table 2** Water analysis from water sample of agriculture research field in Esfahan

| TDS (mg/L) | SO <sub>4</sub> (meq/L) | HCO <sub>3</sub> (meq/L) | CO <sub>3</sub> (meq/L) | Cl <sup>-</sup> (meq/L) | SAR | Na <sup>+</sup> (meq/L) | Mg <sup>++</sup> (meq/L) | Ca <sup>++</sup> (meq/L) | EC (dS/m) | pH   |
|------------|-------------------------|--------------------------|-------------------------|-------------------------|-----|-------------------------|--------------------------|--------------------------|-----------|------|
| 1,680      | 9.0                     | 7.4                      | 0.0                     | 9.8                     | 6.2 | 14.9                    | 5.0                      | 6.4                      | 2.1       | 7.65 |

**Note:** TDS = total dissolved solids, SAR = sodium adsorption ratio, EC = electrical conductivity

### Climatic Measurements

An automatic weather station was installed in the experimental field to record daily air temperature and rainfall during the growing period. Long term average precipitation is 150 mm and this area was semi-arid. Maximum temperature, minimum temperature and relative air humidity values

are shown in Table 3. The mean maximum and minimum temperatures were 33.8 and 13.9°C in 2009, respectively. In 2010, the mean maximum and minimum temperatures during the growing season were 34.1 and 14.2°C, respectively. In 2009 and 2010, relative air humidity was 39.2 and 39.4%, respectively (Table 3).

**Table 3** Changes in mean temperature based on centigrade and relative air humidity during growing season in 2009 and 2010

| Year                       | Maximum temperature (°C) | Minimum temperature (°C) | Relative air humidity |
|----------------------------|--------------------------|--------------------------|-----------------------|
| 2009                       |                          |                          |                       |
| 22 May to 21 June          | 31.5                     | 14.0                     | 45.0                  |
| 22 June to 22 July         | 35.5                     | 18.0                     | 41.0                  |
| 23 July to 22 August       | 39.0                     | 18.7                     | 31.0                  |
| 23 August to 22 September  | 34.1                     | 12.5                     | 40.0                  |
| 23 September to 22 October | 29.0                     | 6.3                      | 39.0                  |
| Mean during season         | 33.8                     | 13.9                     | 39.2                  |
| 2010                       |                          |                          |                       |
| 22 May to 21 June          | 32.0                     | 14.5                     | 44.0                  |
| 22 June to 22 July         | 36.0                     | 18.5                     | 43.0                  |
| 23 July to 22 August       | 38.5                     | 19.2                     | 30.3                  |
| 23 August to 22 September  | 34.2                     | 12.3                     | 41.0                  |
| 23 September to 22 October | 30.0                     | 6.5                      | 38.7                  |
| Mean during season         | 34.1                     | 14.2                     | 39.4                  |

### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using a statistical analysis system, followed by Duncan's multiple range test and the significant differences were considered at  $P < 0.05$  by MSTAT-C software (version 2.10).

## RESULTS AND DISCUSSION

### Plant Height

Plant height was not significantly influenced by both year and nitrogen fertilizer. However, the effect of plant density on this experimental trait was meaningful ( $P < 0.01$ ). Interaction between year and plant density and interaction between year and nitrogen significantly affected plant height. Furthermore, this trait was markedly influenced by the interaction between plant density and nitrogen fertilizer, and interaction among all treatments ( $P < 0.01$ ; Table 4). Although the maximum plant height was achieved in 2009 (193.8 cm), there was no significant difference between 2009 and

2010. The highest plant height was obtained for 400,000 plants/ha (209.5 cm), followed by 350,000, 250,000 and 300,000 plants/ha, respectively. In agreement with the result of this study, Raei and Sharifi (2009) showed that plant height was increased as plant density increased. On the contrary, Cho *et al.* (2004) showed an increase in plant height as plant density decreased. In some cases, high plant density in specific space causes the lack of nutrition, space and fertilizer due to improper growth (Snider *et al.*, 2012). The highest plant height is good agronomical management to achieve more biomass in forage crops. Also, high plant height is because of more biosynthesis of plant growth substance (IAA) in shading environment. In this trial, high plant density was the main reason for interspecific competition and also translocation of assimilates into consumptive aerial organs. On the one hand, the application of 80 kg N/ha obtained the maximum plant height (191.6 cm). On the other hand, there were no significant differences between 80 kg N/ha and other treatments (Table 6).

**Table 4** Analysis of variance for plant height, number of leaves per plant, number of tillers per plant, stem diameter, leaf area index, fresh forage yield and dry forage yield

| Source of variation             | df | Plant height           | Number of leaves per plant | Number of tillers per plant | Stem diameter        | Leaf area index     | Fresh forage yield     | Dry forage yield    |
|---------------------------------|----|------------------------|----------------------------|-----------------------------|----------------------|---------------------|------------------------|---------------------|
| Year (Y)                        | 1  | 9,135.63 <sup>ns</sup> | 0.872 <sup>ns</sup>        | 2.660 <sup>ns</sup>         | 71.088*              | 3.949 <sup>ns</sup> | 1,075.61 <sup>ns</sup> | 14.749*             |
| Replication × Year (Error a)    | 3  | 277.43                 | 0.255**                    | 0.852 <sup>ns</sup>         | 17.540 <sup>ns</sup> | 0.417 <sup>ns</sup> | 749.32 <sup>ns</sup>   | 4.371 <sup>ns</sup> |
| Plant density (D)               | 3  | 5,853.75**             | 0.418**                    | 4.442**                     | 96.016**             | 3.230**             | 50,456.86**            | 163,100**           |
| Year × Plant density            | 3  | 2,635.60**             | 0.147*                     | 1.871 <sup>ns</sup>         | 36.982*              | 1.074*              | 719.68 <sup>ns</sup>   | 8.191 <sup>ns</sup> |
| Error b                         | 12 | 412.90                 | 0.035                      | 0.569                       | 7.806                | 0.277               | 276.66                 | 2.514               |
| Nitrogen (N)                    | 3  | 235.85 <sup>ns</sup>   | 0.120**                    | 0.315 <sup>ns</sup>         | 1.688 <sup>ns</sup>  | 0.181**             | 1,079.37**             | 14.045*             |
| Year × Nitrogen                 | 3  | 473.01**               | 0.229**                    | 0.118 <sup>ns</sup>         | 0.753 <sup>ns</sup>  | 0.087 <sup>ns</sup> | 70.80**                | 27.750**            |
| Plant density × Nitrogen        | 9  | 938.63**               | 0.106**                    | 0.135 <sup>ns</sup>         | 6.825*               | 0.047 <sup>ns</sup> | 46.45**                | 3.454 <sup>ns</sup> |
| Year × Plant density × Nitrogen | 9  | 426.16**               | 0.086**                    | 0.107 <sup>ns</sup>         | 3.354 <sup>ns</sup>  | 0.024 <sup>ns</sup> | 14.52 <sup>ns</sup>    | 2.742 <sup>ns</sup> |
| Error c                         | 48 | 141.63                 | 0.018                      | 0.127                       | 3.113                | 0.031               | 10.32                  | 3.635               |

**Note:** \*significant at  $\alpha = 0.05$  in F-tests, \*\*significant at  $\alpha = 0.01$  in F-tests, ns = not significant



### Number of Leaves per Plant

There was not any significant difference for number of leaves per plant, between 2009 and 2010. On the contrary, number of leaves per plant was significantly influenced by not only plant density, but also nitrogen fertilizer ( $P < 0.01$ ). The effects of interaction between year and plant density ( $P < 0.05$ ) and interaction between year and nitrogen fertilizer ( $P < 0.01$ ) on this trait were significant. Moreover, plant density and nitrogen fertilizer interaction effect on this trait was also meaningful ( $P < 0.01$ ; Table 4). The higher value of number of leaves per plant was related to 400,000 plants/ha (11.15), compared with other treatments. Indeed, planting at the density of 400,000 plants/ha had no significant differences with 350,000 plants/ha for number of leaves per plant, but had significant differences with 250,000 and 300,000 plants/ha. Cho *et al.* (2004) explained that as plant density increased, the number of leaves decreased. The maximum and the minimum values of number of leaves per plant were 11.04 and 10.88, which were obtained for control treatment (0 kg N/ha) and 160 kg N/ha, respectively (Table 6).

### Number of Tillers per Plant

Year and nitrogen fertilizer effects on number of tillers per plant were not significant. Furthermore, interaction between year and plant density, interaction between year and nitrogen, and plant density and nitrogen interaction had no significant influence on this trait. However, plant density effect on this trait was meaningful ( $P < 0.01$ ; Table 4). Moosavi (2012) also showed the significant effect of plant density on number of tillers per plant. The highest degree of number of tillers per plant was obtained in 2010 (2.91) and control treatment (2.87), which had no significant differences with other treatments. A higher value of this trait was obtained when planted at a density of 250,000 plants/ha (3.28), than those of other treatments. The only significant difference was related to the difference between 250,000 and 400,000 plants/ha (Table 6). An increase in plant

density decreased number of tillers per plant and stem diameter (Moosavi, 2012). Lower tillers were produced in a high plant population due to less translocation of assimilates towards lower parts of the plants (Shahrajabian *et al.*, 2011).

### Stem Diameter

Stem diameter was significantly influenced by year ( $P < 0.05$ ), however, nitrogen fertilizer effect on this trait was not meaningful. The effect of plant density on stem diameter was significant ( $P < 0.01$ ). Interaction between year and plant density, and interaction between plant density and nitrogen fertilizer on this trait was significant ( $P < 0.05$ ) as shown in Table 4. Moosavi (2012) also reported the significant effect of plant density on main stem diameter. The higher value of stem diameter (19.13 mm) was related to 2010, than that of 2009. The maximum stem diameter was obtained when planted forage sorghum at density of 250,000 plants/ha (20.56 mm) while the minimum stem diameter was found at 400,000 plants/ha (15.81 mm), which had significant difference with each other. Ayub *et al.* (2003) also reported a decrease in stem diameter under the influence of high plant density. In high plant density, because of interspecific competition and lack of nutrition, stem diameter was thinner than normal and optimum plant density (Shahrajabian *et al.*, 2011; Moosavi, 2012; Snider *et al.*, 2012). The highest degree of stem diameter was related to control treatment (18.63 mm), followed by providing nitrogen fertilizer at 80, 240 and 160 kg N/ha, respectively (Table 6).

### Leaf Area Index (LAI)

Both plant density and nitrogen fertilizer had a significant influence on LAI ( $P < 0.01$ ). However, LAI was not significantly influenced by the interaction between year and nitrogen fertilizer, and interaction between plant density and nitrogen fertilizer (Table 4). There was not any significant difference in LAI between 2009 and 2010. The highest value of LAI (9.22), which had a significant difference with other treatments was achieved

when planted forage sorghum at density of 400,000 plants/ha. Furthermore, all other differences among treatments were not meaningful. Tabo *et al.* (2002) also stated that LAI increased with increasing plant density. Application of 240 kg N/ha had obtained the highest LAI (8.81), and its differences with 80 and 160 kg N/ha were not significant. The lowest one was related to control treatment (Table 6). When the economical aim is the production of forage crops, a suitable leaf area index besides high plant height is so important (Shahrajabian *et al.*, 2011). An increase in plant density on the surface units leads to a reduction in stem diameter and tillers, but it can maximize plant height and LAI (Khalili Moheleh *et al.*, 2007).

### Fresh Forage Yield

Fresh forage yield was significantly influenced by plant density and nitrogen fertilizer ( $P < 0.01$ ), however, the effect of year on this trait was not meaningful. Interaction between year and nitrogen fertilizer, and interaction between plant density and nitrogen fertilizer on fresh forage yield were significant ( $P < 0.01$ ; Table 4). Fresh forage yield in 2009 was higher than in 2010, even though no significant difference was found between treatments. Fresh forage yield was significantly increased as plant density increased from 250,000 to 400,000 plants/ha (Table 6). Moreover, all differences among treatments were significant. The highest value of fresh forage yield was obtained for applying 240 kg N/ha (121 t/ha), followed by 160, 80 and 0 kg N/ha (control treatment). The proper usage of nitrogen and plant density assure crop yield and sustainable productivity (Khalili Moheleh *et al.*, 2007; Li *et al.*, 2011; Shahrajabian *et al.*, 2011; Amini *et al.*, 2012; Becquer *et al.*, 2012; Jothimani, 2012).

### Dry Forage Yield

The effects of year and nitrogen fertilizer on dry forage yield were significant ( $P < 0.05$ ). Indeed, plant density effect on dry forage yield was meaningful ( $P < 0.01$ ). Marsalis *et al.* (2010) reported no effect of plant population and nitrogen rates on dry matter. Among all interactions, just

interaction between year and nitrogen fertilizer had a significant effect on dry forage yield ( $P < 0.01$ ) (Table 4). A significant difference was found between dry forage yield in 2009 (7.93 t/ha) and 2010 (8.71 t/ha). The highest dry forage yield was achieved in 400,000 plants/ha, followed by 350,000, 300,000 and 250,000 plants/ha, respectively. The difference in dry forage yield between 350,000 and 400,000 plants/ha was not significant. The maximum and the minimum dry forage yield was related to 160 kg N/ha (8.97 t/ha) and control treatment (7.33 t/ha) respectively which had significant difference with each other. On the contrary, there were not any significant differences between using 160 kg N/ha and other treatments (80 and 240 kg N/ha). Although, sorghum plants use N nutrients more efficiently than most crops and more tolerant to drought than corn plants, N deficiency suppressed plant growth and dry matter accumulation (Zhao *et al.*, 2005; Shahrajabian *et al.*, 2011). Improving sorghum dry matter may be promoted by improving growth factors, such as plant density and nitrogen fertilizer (Gutte and Karanjikar, 2007; Shahrajabian *et al.*, 2011).

### Panicle Length

Plant density and nitrogen fertilizer had significant effects on panicle length ( $P < 0.01$ ), but this trait was not markedly affected by year. Panicle length was significantly influenced by interactions namely, interaction between year and plant density, interaction between year and nitrogen fertilizer, and interaction between plant density and nitrogen fertilizer (Table 5). There was not any significant difference in terms of panicle length between years. The highest panicle length was related to 300,000 plants/ha (27.03 cm), followed by 350,000, 250,000 and 400,000 plants/ha, respectively. There were significant differences between 400,000 plants/ha with all treatments. Soleymani *et al.* (2012a) also explained that the plant density treatments differed significantly between themselves in panicle length. The maximum and the minimum panicle length was related to applying 240 and 0 kg N/ha, which was 24.40 and 20.57 cm, respectively (Table 7).



**Table 5** Analysis of variance for panicle length, panicle width, number of seeds per panicle, a thousand seed weight, seed yield, prussic acid, protein percentage of the first cut and ash percentage of the first cut

| Source of variation             | df | Panicle length       | Panicle width       | Number of seeds per panicle | A thousand seed weight | Seed yield          | Prussic acid          | Protein percentage of the first cut | Ash percentage of the first cut |
|---------------------------------|----|----------------------|---------------------|-----------------------------|------------------------|---------------------|-----------------------|-------------------------------------|---------------------------------|
| Year (Y)                        | 1  | 1.153 <sup>ns</sup>  | 0.940 <sup>ns</sup> | 409.350 <sup>ns</sup>       | 12.615 <sup>ns</sup>   | 20.841*             | 118.810 <sup>ns</sup> | 0.389**                             | 0.010 <sup>ns</sup>             |
| Replication × Year (Error a)    | 3  | 0.0001               | 0.003               | 0.0001                      | 0.0001                 | 0.0001              | 0.141                 | 0.007                               | 0.0001                          |
| Plant density (D)               | 3  | 355.808**            | 78.180**            | 1,972,109.956*              | 0.060 <sup>ns</sup>    | 3.228*              | 6.524 <sup>ns</sup>   | 0.583**                             | 1.084*                          |
| Year × Plant density            | 3  | 0.009**              | 0.008*              | 1.967 <sup>ns</sup>         | 0.0002 <sup>ns</sup>   | 0.027 <sup>ns</sup> | 6.310 <sup>ns</sup>   | 0.275**                             | 0.157 <sup>ns</sup>             |
| Error b                         | 12 | 0.0001               | 0.002               | 0.0001                      | 0.0001                 | 0.0001              | 1.309                 | 0.013                               | 0.0002                          |
| Nitrogen (N)                    | 3  | 73.270**             | 9.350**             | 6,261,606.591**             | 2.550*                 | 62.307*             | 2.931 <sup>ns</sup>   | 0.361**                             | 0.039 <sup>ns</sup>             |
| Year × Nitrogen                 | 3  | 0.002**              | 0.003 <sup>ns</sup> | 6.247 <sup>ns</sup>         | 0.006 <sup>ns</sup>    | 0.512 <sup>ns</sup> | 1.438 <sup>ns</sup>   | 0.518**                             | 0.059 <sup>ns</sup>             |
| Plant density × Nitrogen        | 9  | 0.917**              | 0.219**             | 795,853.432 <sup>ns</sup>   | 0.051 <sup>ns</sup>    | 0.098 <sup>ns</sup> | 2.885 <sup>ns</sup>   | 0.265**                             | 0.137 <sup>ns</sup>             |
| Year × Plant density × Nitrogen | 9  | 0.0002 <sup>ns</sup> | 0.003 <sup>ns</sup> | 0.795 <sup>ns</sup>         | 0.0001 <sup>ns</sup>   | 0.001 <sup>ns</sup> | 3.567 <sup>ns</sup>   | 0.227**                             | 0.127 <sup>ns</sup>             |
| Error c                         | 48 | 0.0001               | 0.002               | 0.0001                      | 0.0002                 | 0.0002              | 3.333                 | 0.044                               | 0.0002                          |

**Note:** \*significant at  $\alpha = 0.05$  in F-tests, \*\*significant at  $\alpha = 0.01$  in F-tests, ns = not significant

### Panicle Width

Panicle width was significantly affected by plant density and nitrogen fertilizer ( $P < 0.01$ ). However, it did not markedly influence by year. This trait also was not significantly influenced by interactions between treatments (Table 5). No significant difference was found in terms of panicle width between 2009 and 2010. The maximum panicle width was 13.11 cm, which was related to 300,000 plants/ha. All differences among plant density were significant. On the one hand, application of 240 kg N/ha had obtained the highest panicle width (11.97 cm), which had significant differences with 0 and 80 kg N/ha. On the other hand, the lowest panicle width, which was 10.55 cm and occurred for control treatment, had significant differences with all treatments, except treatment with 80 kg N/ha (Table 7).

### Number of Seeds per Panicle

Number of seeds per panicle was not markedly influenced by year, however, the effect of plant density on number of seeds per panicle was meaningful ( $P < 0.05$ ). Fanaei *et al.* (2002), Baradaran *et al.* (2007) and Soleymani *et al.* (2011) reported that number of seeds per panicle in grain sorghum was affected by plant density. Nitrogen fertilizer effect on this trait was also significant. Interactions had no significant effect on this trait (Table 5). Although the maximum number of seeds per panicle was found in 2010 (2,069), it had no significant difference with 2009. The number of seeds per panicle was decreased significantly from 250,000 to 400,000 plants/ha, furthermore, all differences were significant. In high plant density, the number of seeds per panicle decreases, but because of having more plants, compensate for producing appropriate seed yield is done (Shahrajabian *et al.*, 2011). The maximum and the minimum number of seeds per panicle were obtained for 80 (2,581) and 240 kgN/ha (1,834), respectively, which had significant differences with other treatments (Table 7). The results of this experiment are different from Soleymani *et al.* (2011) who reported that

the nitrogen treatments did not differ significantly between themselves in the number of seeds per panicle treatments. Asghari *et al.* (2006) indicated that with increasing levels of nitrogen, the number of seeds per panicle was increased.

### A Thousand Seed Yield

Plant density and year had not markedly effect on a thousand seed weight. However, this trait was significantly affected by nitrogen fertilizer ( $P < 0.05$ ). None of the interactions had a significant influence on a thousand seed yield (Table 5). In contrast, Soleymani *et al.* (2011) reported that a thousand seed yield was not markedly affected by plant density, but in the experiment of Fanaei *et al.* (2002), a thousand seed yield was markedly influenced by plant density. The highest value of a thousand seed weight was obtained in 2010 (8.01 g) and at a density of 250,000 plants/ha (7.72 g). Moreover, both treatments had no significant differences from other treatments. A low plant density can be compensated by a high number of grains per panicle and high weight of the grain (Mallikarjun *et al.*, 1997; Fanaei *et al.*, 2002). From the application of 0 (control treatment) to 160 kg N/ha, a thousand seed weight was increased significantly, but after that, it decreased, and the lowest one was 7.29 g, which had meaningful differences with other treatments (Table 7). However, Soleymani *et al.* (2011) reported that the nitrogen treatments did not differ significantly between treatments in number of a thousand seed weight.

**Table 6** Mean comparison for plant height, number of leaves per plant, number of tillers per plant, stem diameter, leaf area index, fresh forage yield and dry forage yield

| Treatment                 | Plant height (cm)   | Number of leaves per plant | Number of tillers per plant | Stem diameter (mm)  | Leaf area index    | Fresh forage yield (t/ha) | Dry forage yield (t/ha) |
|---------------------------|---------------------|----------------------------|-----------------------------|---------------------|--------------------|---------------------------|-------------------------|
| Year                      |                     |                            |                             |                     |                    |                           |                         |
| 2009                      | 193.8               | 11.07                      | 2.57                        | 17.41 <sup>a</sup>  | 8.92               | 117.00                    | 7.93 <sup>a</sup>       |
| 2010                      | 184.8               | 10.08                      | 2.91                        | 19.13 <sup>b</sup>  | 8.51               | 116.30                    | 8.71 <sup>b</sup>       |
| Plant density (plants/ha) |                     |                            |                             |                     |                    |                           |                         |
| 250,000                   | 177.6 <sup>b</sup>  | 10.92 <sup>b</sup>         | 3.28 <sup>a</sup>           | 20.56 <sup>a</sup>  | 8.38 <sup>b</sup>  | 62.54 <sup>d</sup>        | 5.06 <sup>c</sup>       |
| 300,000                   | 176.3 <sup>b</sup>  | 10.84 <sup>b</sup>         | 2.81 <sup>ab</sup>          | 18.94 <sup>ab</sup> | 8.52 <sup>b</sup>  | 97.99 <sup>c</sup>        | 7.51 <sup>b</sup>       |
| 350,000                   | 193.7 <sup>ab</sup> | 11.00 <sup>ab</sup>        | 2.63 <sup>ab</sup>          | 17.79 <sup>bc</sup> | 8.74 <sup>b</sup>  | 133.00 <sup>b</sup>       | 9.75 <sup>a</sup>       |
| 400,000                   | 209.5 <sup>a</sup>  | 11.15 <sup>a</sup>         | 2.24 <sup>b</sup>           | 15.81 <sup>c</sup>  | 9.22 <sup>a</sup>  | 169.30 <sup>a</sup>       | 10.97 <sup>a</sup>      |
| Nitrogen (kg/ha)          |                     |                            |                             |                     |                    |                           |                         |
| 0                         | 191.4               | 11.04 <sup>a</sup>         | 2.87                        | 18.63               | 8.60 <sup>b</sup>  | 106.60 <sup>c</sup>       | 7.33 <sup>b</sup>       |
| 80                        | 191.6               | 11.01 <sup>ab</sup>        | 2.79                        | 18.24               | 8.70 <sup>ab</sup> | 114.70 <sup>b</sup>       | 8.11 <sup>a</sup>       |
| 160                       | 184.8               | 10.88 <sup>b</sup>         | 2.61                        | 17.99               | 8.75 <sup>a</sup>  | 120.50 <sup>a</sup>       | 8.97 <sup>a</sup>       |
| 240                       | 189.4               | 10.99 <sup>ab</sup>        | 2.69                        | 18.24               | 8.81 <sup>a</sup>  | 121.00 <sup>a</sup>       | 8.87 <sup>a</sup>       |

**Note:** Common letters within each column do not differ significantly in F-test

### Seed Yield

Seed yield was significantly influenced by year, plant density and nitrogen fertilizer ( $P < 0.05$ ) (Table 5). Fanaei *et al.* (2002), Javanmard *et al.* (2006), Ould Ahmed *et al.* (2007), Jia *et al.* (2010) and Soleymani *et al.* (2011) reported the significant influence of plant densities on grain yield of sorghum. Soleymani *et al.* (2011) reported the significant influence of nitrogen on seed yield. The highest value of seed yield was obtained in 2010 (5.61 t/ha), which had significant difference with 2009. Seed yield was decreased significantly from 5.58 to 4.73 t/ha when plant density increased from 250,000 to 400,000 plants/ha. Furthermore, all differences among treatments were significant. In agreement with the result of this experiment, Fanaei *et al.* (2002) and Gutte and Karanjikar (2007) reported that the grain yield of sorghum decreased with higher plant densities, but Javanmard *et al.* (2006) concluded that the maximum grain yield was obtained at the highest plant density. Berenguer and Faci (2001) indicated that a high plant density did not present

productive advantages in the sorghum grain yield. The maximum and the minimum seed yield were achieved in the application of 160 (7.18 t/ha) and 0 kg N/ha (3.48 t/ha), respectively. There were significant differences among treatments (Table 7). Grain yield is probably the most important characteristic on which to base nitrogen fertilizer application and selection of appropriate plant density (Khoshkharam *et al.*, 2010; Shahrajabian *et al.*, 2011; Soleymani *et al.*, 2011; Soleymani *et al.*, 2012a). Asghari *et al.* (2006) indicated that with increasing levels of nitrogen, grain yield was increased. Variation in grain yield was related to the number of seed per panicle and a thousand seed weight. Balasubramanian and Ramamoorthy (1996) reported that grain yield was improved by increasing nitrogen fertilizer application and decreasing plant density. Different grain yields in different levels of nitrogen and planting densities were due to the increased number of grains per basic panicle and increased number of panicles per m<sup>2</sup> (Jalali and Bahrani, 2001).

**Table 7** Mean comparison for panicle length, panicle width, number of seeds per panicle, a thousand seed weight, seed yield, prussic acid, protein percentage of the first cut and ash percentage of the first cut

| Treatment                 | Panicle length (cm) | Panicle width (cm) | Number of seeds per panicle | A thousand seed weight (g) | Seed yield (t/ha) | Prussic acid (ppm) | Protein of the first cut (%) | Ash of the first cut (%) |
|---------------------------|---------------------|--------------------|-----------------------------|----------------------------|-------------------|--------------------|------------------------------|--------------------------|
| Year                      |                     |                    |                             |                            |                   |                    |                              |                          |
| 2009                      | 17.27               | 11.15              | 2,065                       | 7.29                       | 4.76 <sup>b</sup> | 232.0              | 11.07 <sup>b</sup>           | 11.97                    |
| 2010                      | 18.76               | 11.35              | 2,069                       | 8.01                       | 5.61 <sup>a</sup> | 229.7              | 10.94 <sup>a</sup>           | 11.95                    |
| Plant density (plants/ha) |                     |                    |                             |                            |                   |                    |                              |                          |
| 250,000                   | 21.59 <sup>c</sup>  | 11.07 <sup>c</sup> | 2,484 <sup>a</sup>          | 7.72                       | 5.58 <sup>a</sup> | 230.5              | 10.93 <sup>b</sup>           | 12.16 <sup>a</sup>       |
| 300,000                   | 27.03 <sup>a</sup>  | 13.11 <sup>a</sup> | 2,017 <sup>b</sup>          | 7.66                       | 5.27 <sup>b</sup> | 230.6              | 10.83 <sup>b</sup>           | 12.10 <sup>b</sup>       |
| 350,000                   | 23.52 <sup>b</sup>  | 11.98 <sup>b</sup> | 1,924 <sup>c</sup>          | 7.62                       | 4.98 <sup>c</sup> | 231.6              | 11.19 <sup>a</sup>           | 11.70 <sup>d</sup>       |
| 400,000                   | 17.80 <sup>d</sup>  | 8.84 <sup>d</sup>  | 1,844 <sup>d</sup>          | 7.61                       | 4.73 <sup>d</sup> | 230.7              | 11.07 <sup>a</sup>           | 11.87 <sup>c</sup>       |
| Nitrogen (kg/ha)          |                     |                    |                             |                            |                   |                    |                              |                          |
| 0                         | 20.57 <sup>d</sup>  | 10.55 <sup>a</sup> | 1,834 <sup>c</sup>          | 7.51 <sup>c</sup>          | 3.48 <sup>d</sup> | 230.8              | 10.89 <sup>b</sup>           | 11.90                    |
| 80                        | 21.53 <sup>c</sup>  | 10.97 <sup>a</sup> | 2,581 <sup>a</sup>          | 7.78 <sup>b</sup>          | 5.59 <sup>b</sup> | 231.0              | 11.02 <sup>ab</sup>          | 11.98                    |
| 160                       | 22.44 <sup>b</sup>  | 11.51 <sup>b</sup> | 2,388 <sup>b</sup>          | 8.04 <sup>a</sup>          | 7.18 <sup>a</sup> | 230.4              | 11.17 <sup>a</sup>           | 11.98                    |
| 240                       | 24.40 <sup>a</sup>  | 11.97 <sup>b</sup> | 1,466 <sup>d</sup>          | 7.29 <sup>d</sup>          | 4.31 <sup>c</sup> | 231.2              | 10.94 <sup>b</sup>           | 11.97                    |

**Note:** Common letters within each column do not differ significantly in F-test

### Prussic Acid

Not only year, plant density and nitrogen fertilizer, but also other interactions had not any significant influence on prussic acid and no trends were found (Table 5). The higher value of prussic acid was related to 2009 compared with 2010. The maximum number of prussic acids was obtained for 350,000 plants/ha, followed by 400,000, 300,000 and 250,000 plants/ha. Application of 240 kg N/ha had obtained the highest number of prussic acids. All differences among treatments were not meaningful (Table 7). However, Bahrani and Ghenatghehstani (2004) reported that increasing nitrogen rates increased forage prussic acid.

### Protein Percentage of the First Cut

Protein percentage of the first cut was significantly influenced by year, plant density and Nitrogen fertilizer ( $P < 0.01$ ). This experimental trait was also significantly affected by the interactions (Table 5). However, Khalili Moheleh *et al.* (2007) reported that year and plant density did not affect protein percentage of the first cut. Protein percentage of the first cut in 2009 was significantly higher than in 2010. The higher value of protein percentage was related to 350,000 plants/ha (11.19%), than those of other treatments, which had significant differences with 250,000 and 300,000 plants/ha. The maximum and the minimum protein percentage were achieved

in the application of 160 kg N/ha (11.17%) and control treatment (10.89%), respectively. Ahmed (2004), and Bahrani and Ghenatghestani (2004) reported that increasing plant density significantly decreased protein percentage. Although 160 kg N/ha had no significant differences with 80 kg N/ha, its differences were significant with other treatments (Table 7). Glamoclija *et al.* (2011) reported that by increasing the nitrogen nutrition, the total protein content in samples was increased. Jalali and Bahrani (2001) concluded that nitrogen application increased the grain protein content.

#### Ash Percentage of the First Cut

The effects of year and nitrogen fertilizer on ash percentage of the first cut were not meaningful. However, it was significantly affected by plant density ( $P < 0.05$ ), but Khalili Moheleh *et al.* (2007) reported that plant density had no significant effect on ash percentage. Ash percentage was not markedly affected by any interactions between treatments (Table 5). There was no significant difference between 2009 and 2010. Ash percentage was decreased significantly with increasing plant density from 250,000 to 400,000 plants/ha, moreover, all differences were significant. Ahmed (2004) reported that ash percentage increased significantly by increasing plant density. The maximum ash percentage was related to the application of both 80 and 160 kg N/ha, which had no differences from other treatments (Table 7).

## CONCLUSIONS

The higher stem diameter and dry forage yield were obtained for 2010 than compared to those data in 2009. The maximum plant height, number of leaves per plant, LAI, fresh forage yield and dry forage yield was achieved in 400,000 plants/ha. Planting at the density of 250,000 plants/ha obtained the highest number of seeds per panicle (2,484), a thousand seed weight (7.72 g) and seed yield (5.58 t/ha). There is no difference in prussic acid among treatments. The highest protein and ash percentages of the first cut were found in 350,000 and 250,000 plants/ha, respectively. There were not any significant differences in terms of plant height, number of tiller per plant, stem diameter, prussic acid and ash percentage between the levels of nitrogen fertilizers. The highest fresh forage yield and dry forage yield was achieved in 240 and 160 kg N/ha, respectively. An adequate supply of N to crops is fundamental to optimize crop yield and crop production. The higher a thousand seed weight and seed yield obtained for 160 kg N/ha than those of other treatments, which were 8.04 g and 7.18 t/ha, respectively. Grain yield is probably the most important characteristic on which to base nitrogen fertilizer application and selection of appropriate plant density. Moreover, the application of 160 kg N/ha had obtained the highest amount of protein percentage of the first cut (11.17%). Also, an application of nitrogen fertilizer and agronomical management such as select suitable plant density can produce the appropriate dry matter and reduce the risk of forage crop production.

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