ORIGINAL PAPER

Yield and biochemical properties of grain sorghum (*Sorghum bicolor* **L. Moench) afected by nano‑fertilizer under feld drought stress**

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Abstract

The use of new and efficient methods for fertilizing plants is of economic and environmental significance. A 2-year field experiment was conducted to investigate the efect of nano (micro and macro) and chemical fertilizers (N, P, K) on grain sorghum yield and biochemical properties including phosphorus (P), calcium (Ca), protein, crude fat and tannin contents under drought stress. The experiment was setup in a split plot randomized complete block design with four replications. The drought stress treatment (main plots) consisted of stoppage of irrigation before (D1) and after fowering (D2), and control (normal) irrigation (D3). The single and the combined use of nano- and chemical fertilizers including control (without fertilization) were used as the sub plots. In control (non-stress) conditions, there were not any diferences between nano-fertilizer with chemical fertilizer on nutrient uptake (P and Ca), crop yield, and seed protein content. However, nanofertilizer enhanced sorghum yield and biochemical properties in drought stress conditions. In D1, grain yield (6240 kg ha⁻¹), and crude fat (5.29%), and in D2, P uptake (3498.87 mg kg⁻¹), protein (21.5%) and tannin (0.373%) were most affected by nano- and chemical fertilizers. Control irrigation and chemical fertilization resulted in the highest grain yield, which was not significantly different from control irrigation with macro- and micro-fertilizer (6213.3 kg ha⁻¹). The tested nano-fertilizers can be used as a suitable source of nutrients for planting sorghum plants in the arid- and semi-arid areas of the world, with economic and environmental benefts.

Keywords Crude fat · Flowering · Protein percentage · Tannin · Water deficiency

Introduction

Grain sorghum (*Sorghum bicolor*), as a resistant plant to drought and salinity and with high nutritional value, is one of the important components of poultry and livestock nutrition, especially in arid and semi-arid regions of the world. This plant with a 95% nutritional similarity with corn is highly considered by poultry breeders for nutritional purposes (Obizoba [1988](#page-7-0)). Sorghum is used all over the world in various forms such as baked bread, grilled, alcoholic and nonalcoholic beverages, etc. Grain sorghum, like other grains, is a good source of starch and protein, and as a gluten-free grain, can be used to treat celiac disease (Ratnavathi and Komala [2016\)](#page-8-0). Phenolic compounds, favonoids and various antioxidants of grain sorghum make it more useful for nutritional applications. Accordingly, grain sorghum can reduce the risks of various diseases including cancer, rheumatoid, etc., by reducing the number of free radicals (Vanamala et al. [2018](#page-8-1); Rashawn et al. [2021\)](#page-8-2).

Sorghum contains vitamin B, and its mineral content is highly variable (Kulamarva et al. [2009](#page-7-1)). In crude sorghum, the total oil composition ranges from 3.91 to 3.58%, and about 84% of which contains oleic and linoleic acid. The tannin content in sorghum pericarp is one of the factors afecting sorghum nutritional values including digestibility and metabolism energy. The presence of tannins in grain sorghum reduces its digestibility and nutritional quality. Dense tannin is found in some species of sorghum, and on the basis of tannin presence into the inner and outer shell, sorghum is classifed into diferent types (Queiroz et al. [2018;](#page-8-3) Shirmohammadli et al. [2018](#page-8-4)).

The nutritional application of grain sorghum is determined by the amount of grain protein and phenolic

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compounds (especially tannins), which are afected by different agronomic factors such as application of fertilizers. Although phenolic compounds have some benefts such as enhancing plant tolerance in intense sunlight, resistance to drought stress and prevention of pre-harvest germination, they also negatively affect protein digestion (Zhao et al. [2020](#page-8-5)) and nutrient uptake (Lin et al. [2016](#page-7-2)) in diferent plant tissues. Despite being resistant to drought stress, the physiological properties of grain sorghum are negatively afected by biotic and abiotic stresses through the production of reactive oxygen species. These species reduce the concentration of photosynthetic pigments and disrupt the metabolic balance of plant (Proietti et al. [2015](#page-8-6); Getachew et al. [2016](#page-7-3)).

Diferent types of fertilizers help the plant in stress conditions by improving the nutritional status of plant. Nutrition, water and their interactions as well as their allocation to the plant afect plant resistance under stress (Sabet and Mortazaeinezhad [2018](#page-8-7); Asadi et al. [2019\)](#page-7-4). Chemical fertilizers have been extensively and globally used in the past six decades to produce sufficient food resources; however, it is not economically and environmentally recommendable at it is subjected to leaching (Miransari and Mackenzie [2015](#page-7-5); Miransari and Smith [2019](#page-7-6)). Accordingly, the use of modern and efficient fertilization techniques such as nanotechnology for efficient fertilizer production to reduce environmental pollution has been proposed, tested and used (Aghajani and Soleymani [2017\)](#page-7-7).

Nanotechnology has provided solutions to increase the value of agricultural crops and to solve environmental issues (Seleiman et al. [2021\)](#page-8-8). The interactions of plant cells with nanoparticles lead to altered plant gene expression and related biological pathways, which ultimately afect plant growth. However, the absorption efficiency and effect of different nanoparticles on the growth and metabolic functions of diferent plants are diferent (Zhao et al. [2017\)](#page-8-9). Nanoparticles are more reactive due to their higher surface area increasing their absorption by plant (Sadeghi et al. [2021](#page-8-10)). The main goal of using nano-fertilizer has been to further reducing fertilizer use and improving plant nutrient efficiency (Raliya et al. [2017](#page-8-11)).

Some researchers believe that nano-fertilizers under environmental fuctuations such as temperature, soil acidity and humidity can help the plant to grow better and because of their controlled uptake are preferable to chemical fertilizer (Raliya et al. [2017](#page-8-11); Guo et al. [2018](#page-7-8)).

Due to specifc climatic conditions (warm and dry and little rainfall) in the province of Sistan-va-Baluchestan, Iran, most plants are subjected to drought stress at diferent stages of their growth and depending on the type and severity of the stress, their yield is afected. Sorghum as a resistant plant to drought stress (Sanjari et al. [2021\)](#page-8-12), with optimum amount of yield and short growing season and easy production is a remarkable crop for the farmers of the region. Large quantities of conventional chemical fertilizers are used for sorghum planting, afecting plant biochemical properties including the nutritional values of cereal grains (Dimkpa et al. [2017](#page-7-9)).

With respect to the above-mentioned details, and because more has yet to be investigated on the use of nano-fertilizer afecting sorghum growth and quality under drought stress, the present research (a 2-year feld experiment) was proposed. The objective was to test the effects of nano (macro+micro)-fertilizers on the yield and the biochemical properties of grain sorghum under drought stress conditions of Sistan-va-Baluchestan province, Iran.

Materials and methods

This experiment was carried out in the summer of 2017 and 2018 at the Agricultural and Natural Resources Research Station of Sistan (Zahak city, Iran) with the eastern latitude of 30.54°, and northern longitude of 61.41° and an altitude of 483 m. The climate of the experimental site is hot and dry, with an average annual temperature of 23 °C and a humidity of 38%. Soil physical and chemical properties were determined using the standard methods (Miransari et al. [2008\)](#page-7-10).

The experiment was set up in a split plot randomized complete block design with four replications with plots measuring 15 m^2 . Main plots included three levels of water stress including 1) before fowering (the feld was irrigated at planting and was re-irrigated at the appearance of the frst flower) (D1)), after flowering (the field was not irrigated after emergence of 50% of fowers until harvest) (D2) and control (regular irrigation) (D3). The single and combined use of micro-and-macro nano- and chemical fertilizers were devoted to the subplots. The nano-fertilizer was sprayed in two diferent stages including the beginning of stemming and two weeks later (before flowering) at 2 g. L^{-1} by a rear sprayer after calibrating at 1 atm.

Diferent growth stages were considered based on the Zadox system. The nano-micro-fertilizer with a balanced combination of iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), calcium (Ca), magnesium (Mg) and sulfur (S), and nano-macro-fertilizer with nitrogen (N), phosphorus (P) and potassium (K) were used for the experiment. Chemical fertilizer (incorporated into the soil) was used as urea (200 kg.ha⁻¹, half before planting and half before fowering), triple superphosphate (250 kg. ha^{-1}) and potassium sulfate (75 kg.ha⁻¹). The plants were harvested after the dough stage, when upper leaves turned yellow. The four middle rows (8 m^2) (ignoring 50-cm margins from the two sides of each row) were harvested, and the grain yield was calculated on the basis of a 12% moisture.

The grains were analyzed for the contents of Ca and P, crude fat, protein and tannin in the laboratory. The percentage of seed fat was calculated according to National Standard No. 10700 with the following details of the sample was weighed. The Soxhlet balloon was brought to a constant weight and weighed. Soxhlet set was then closed, and five grams milled sample was inserted into the cartouche and was treated with N-hexane solvent, and the extraction operation was performed for 6 to 8 h. The solvent was then removed, and the Soxhlet balloon was oven dried and weighed. Using the following equation, the percentage of fat was calculated.

The percentage of seed crude protein was measured by Kjeldahl method and according to the National Iranian Standards Organization 19,052 in the laboratory. Nitrogen content was measured using a fully automated Kjeldahl apparatus including three stages of digestion, distillation and titration. After titration, N (%) was calculated using the following equation (Mossé and Baudet [1983\)](#page-7-11). The conversion coefficient of protein was used for the calculation of crude protein content as a percentage of dry matter mass.

 $f \text{at}(\%) = \frac{\text{secondary weight of balloons} - \text{primary with of balloons}}{\text{sample weight}} \times 100$

Seed P was determined according to National Standard No. 513 using a spectrophotometer at 430 nm and the following equation (the details of measurement are available at request).

$$
\text{Seed } P = \frac{50 \times 0.001 \times w \times 10}{\text{sample weight} \times 10}
$$

W=reading from device.

Seed Ca was obtained according to National Standard Method No. 1–10,701 using the following equation.

$$
Seed Ca = \frac{20.04 \times V \times 0.1 \times 250}{\text{sample weight} \times 10 \times 20}
$$

v=consumed potassium permanganate 0.1 N.

Seed tannin (%) was measured according to National Standard No. 9341 IS IRI 9341 as follows. (1) Tannin was extracted by centrifugation by dimethylformamide and by adding ferric ammonium citrate and ammonia solution to the upper part of the fuid (2) the absorption at 525 nm was determined by spectrometer, and (3) the amount of sample tannin was determined using standard curves and tannic acid.

 $N = \frac{X - 14.008}{W}$

 $X =$ titration reading.

W=sample weight.

Data were analyzed using EXCEL and SAS version 9.2 software, and means were compared by the Duncan's multiple range test at the 5% level of probability.

Results

Analysis of variance

According to the analysis of variance, both drought stress and fertilization significantly ($p \le 0.05$) affected sorghum grain yield, P, Ca, protein, fat, and tannin contents. The interactions of the experimental treatments also signifcantly afected all the measured traits except grain fat percentage $(p \le 0.1)$. The effect of year and its interaction with fertilization was just signifcant on seed tannin (Table [1](#page-2-0)).

Table 1 Analysis of variance indicating the signifcance of the experimental treatments afecting the measured parameters

SV: source of variation, *df* degree of freedom, *P* phosphorous, *Ca* calcium, *CV*: coefficient of variation **Significant at $P \leq 0.01$

Grain yield

P and Ca uptake

The highest grain yield resulted from the control irrigation and chemical fertilizer application (6240 kg/ha) and was not significantly different from control irrigation with macro- and micro-fertilizer (6213.3 kg/ha). Accordingly, both treatments increased seed yield almost equally (Fig. [1a](#page-3-0)). The least seed yield was resulted by D2 without fertilization (2738.8 kg/ha) (Table [2](#page-4-0)).

Treatment D2 resulted in the highest grain P at diferent fertilization levels ranging from 3486.5 to 3498.87 mg kg⁻¹ significantly higher than D1 (3308.62–3358 mg kg⁻¹) and D3 (3411.12–3470.87 mg kg−1) (Fig. [1b](#page-3-0)). Treatment D3 (control) and macro- and micro- (5903.37 mg kg⁻¹) or chemical fertilizer (5894.8 mg kg⁻¹) resulted in the highest grain Ca. However, treatment D2 resulted in the least grain Ca at diferent levels of fertilization (5520.75–5575.25 mg kg^{-1}) (Fig. [1](#page-3-0)c).

Grain yield (kg/ha) A $\mathbf A$ 7000 6000 Grain yield (kg/ha) 5000 $\overline{\text{d}}$ $\overset{d}{I}$ ef ef e Ι $\rm e$ α 4000 Ι I. g h 3000 \Box D1 2000 \blacksquare D₂ 1000 $D3$ \overline{O} **F1 F2 F3 F4 F5 B**4000 a C $\frac{a}{b}$ f C d
I $\frac{d}{L}$ \hat{I} Î Ι 3000 **P (mg/kg)** 2000 \Box D1 \blacksquare D₂ 1000 $D3$ $\mathbf 0$ **F1 F2 F3 F4 F5** $\mathbf C$ **Ca (mg/kg) C**6000 a \overline{a} 5900 5800 Ca (mg/kg) d 5700 Ι e I Ι e gf I, g 5600 $\mathbf I$ $D1$ $\overline{}$ h \overline{h} 5500 \blacksquare D₂ 5400 $D3$ 5300 **F1 F2 F3 F4 F5**

Fig. 1 Grain sorghum: **a** yield, **b** P, and **c** Ca affected by nanoand chemical fertilizer under drought stress. D1 (irrigation before fowering), D2 (irrigation after fowering), D3 (control, normal irrigation). $F1 =$ macro, F2=micro, F3: chemical fertilizer, F4: macro+micro F5=control (without fertilizer). Mean values followed by the same letters are not significantly different at $P \leq 0.05$ using Duncan's multiple range test

Table 2 Treatment affecting the measured parameters

Tannin
0.26d
0.2e
d 255/0
0.193e
0.165f
0.373a
0.126g
0.342 _b
0.33bc
c 0.312c
0.26d
0.215e
0.251d
0.206e
0.163f

D1 (irrigation before fowering), *D2* (irrigation after fowering), *D3* (control, normal irrigation). F1=macro, F2=micro, F3: chemical fertilizer, F4: macro+micro F5=control (without fertilizer). Mean values followed by the same letters are not significantly different at $P \le 0.05$ using Duncan's multiple range test

Grain protein

The highest grain protein was related to D2 and micro (21.5%), not signifcantly diferent from D2 and chemical fertilizer (21.08%), however, signifcantly higher than D1 (13.15–13.81%) and D3 (13.14–13.57%) (Fig. [2](#page-5-0)a).

Crude fat

The highest grain crude fat was related to D1 and macrofertilizer (5.29%) followed by D3 and macro+ micro and D3 without fertilizer application. The least grain crude fat was resulted by D2 at diferent levels of fertilization ranging from 3.3–3.63%. (Fig. [2b](#page-5-0)).

Tannin

D2 and macro resulted in the highest grain tannin (0.373%) , and D2 and micro resulted in the least grain tannin (0.126%). The grain tannin of D1 at diferent fertilization levels was in the range of 0.165–0.26%, and of D3 was in the range of 0.163–0.26% (Fig. [2](#page-5-0)c).

Discussion

The tested nano-fertilizer application enhanced sorghum yield and biochemical properties in drought stress conditions. Grain yield, Ca and crude fat were most afected by nano- and chemical fertilizer in D1, and P, protein and tannin were most afected by nano- and chemical fertilizer in D2.

The highest grain yield was related to D3 and chemical fertilizer (containing N, P and K), which is due to the positive role of N in promoting vegetative growth, soil fertility, chlorophyll concentration and the favorable role of P in stimulating the formation of trioses and carbon dioxide stabilization, as well as the positive efect of K on maintaining photosynthetic activities in an optimal mode. Accordingly, the above-mentioned factors increase grain yield by afecting plant metabolic and physiological processes (de Oliveira et al. [2020](#page-7-12)). The positive efects of N and P chemical fertilizer on sorghum grain yield has also been shown by diferent researchers (Fontes et al. [2017](#page-7-13); Jankowski et al. [2020](#page-7-14)). There was not a signifcant diference between chemical fertilizer with D3+macro+microfertilizer on sorghum grain yield, which is similar to the results of the Rehab et al. [\(2020](#page-8-13)) examining the efect of N, P, K fertilizer and micronutrients on sorghum.

The lowest grain yield was observed by D2 and non-fertilization, indicating sorghum yield vulnerability, at seed flling stage, under drought stress conditions. Dehydration by drought stress, before harvest, can signifcantly reduce crop yield by afecting stomatal opening and decreasing the activity of enzymes in the Kelvin's cycle, and subsequent seed flling in sorghum (Awasthi et al. [2014](#page-7-15); Farooq et al. [2018\)](#page-7-16).

The D1 and D2 stress levels with nano- and chemical fertilizer afected seed protein percentage as the highest was related to $D2 +$ micro (Fig. [2a](#page-5-0)) and the least to $D3 +$ without fertilizer. Noori et al. ([2021\)](#page-7-17) found the least and the highest seed protein contents were realized by optimum irrigation and severe drought stress, respectively. During the seed **Fig. 2** Grain sorghum: **a** protein, **b** fat, and **c** tannin afected by nano- and chemical fertilizer under drought stress. D1 (irrigation before fowering), D₂ (irrigation after flowering), D3 (control, normal irrigation). $F1 =$ macro, $F2 =$ micro, F3: chemical fertilizer, F4: macro+micro F5=control (without fertilizer). Mean values followed by the same letters are not signifcantly diferent at *P*≤0.05 using Duncan's multiple range test

flling period, under drought stress, plant uses the proteinmaking mechanism to deal with the stress.

The presence of Fe, Zn and molybdenum (Mo) in the micro-treatment can significantly affect crop yield, due to the following: (1) the signifcant role of Zn in protein synthesis and improvement of nutritional conditions, (2) the effect of Mo on the activity of nitrogenase and nitrate reductase, and (3) the efects of Fe on plant photosynthesis and protein synthesis, which increases seed protein content. Micronutrients can also enhance seed protein percentage by improving the yield of optical photosystems, especially under drought stress (Dimkpa et al. [2020](#page-7-18); Li et al. [2020a](#page-7-19); Wu et al. [2020](#page-8-14)). In the case of sorghum, high concentration of proline under drought stress can be a good source for seed transfer and can increase seed protein concentration (Nxele et al. [2017](#page-7-20)).

Grain P was the highest in D2 by nano- and chemical fertilizer, not statistically diferent from each other (Dimkpa et al. [2019;](#page-7-21) Aqaei et al. [2020](#page-7-22)). It is likely that under drought stress before harvest, plant increased sugar and proline contents for the osmotic regulation of the roots and increased P uptake resulted in the more allocation of P to the grains. The least grain P also was observed with $D1$ + without fertilization (Zehetner et al. [2018;](#page-8-15) Ghafari et al. [2019](#page-7-23)). Sorghum plants were exposed to water defcit in their vegetative phase, without any fertilizer application, so the absorbed P was not enough for allocation to the grain in later stages.

During the vegetative growth stage, plant consumes all available P to meet plant current need for growth and development; however, during the reproductive phase because the grains are a stronger destination for P uptake, more P is transferred to the grains (Wang et al. [2018](#page-8-16)). There were not any signifcant diferences between the fertilizer treatments, in terms of P uptake, indicating that fertilizer application was not advantageous for increasing sorghum grain P under drought stress before harvest.

Grain Ca was also affected by drought stress and fertilization. The highest grain Ca was observed with D3+macro+micro- and D3+chemical fertilizer. The combined use of micro- and macro-nutrients increased grain nutrient concentration including Ca in sorghum. The least grain Ca was related to $D3$ + control (without) fertilizer, signifcantly diferent from the other treatments. The presence of potassium in chemical fertilizer and macro+micro can reduce calcium uptake under drought stress (Zamani et al. [2020](#page-8-17)). Calcium is mainly transmitted to the root surface by mass flow, and the moisture deficiency limits calcium uptake and transport to the roots. Plant reduces evapotranspiration under drought stress, resulting in the decreased uptake and distribution of calcium in diferent plant tissues (Liang and Zhang [2018\)](#page-7-24). It is also possible that under drought stress conditions, long-term wetting and drying of the soil will release K ions from the clay layers and increase K concentration in the soil, and subsequent K uptake, which decreases Ca uptake (Park et al. [2019](#page-8-18)).

In the present research, grain Ca content in D2 was the least, indicating that the plant uses other mechanisms for stress tolerance rather than increase in Ca content, which can also afect P concentration. According to the results, the treatments with the least Ca content had the highest P content. There is a high probability of antagonistic and synergistic among nutrients in sorghum (Aqaei et al. [2020\)](#page-7-22).

The highest grain fat was related to D1 and D3, and there were not any signifcant diferences among the diferent treatments of fertilization. During the grain flling period, frst protein and then oil is formed; the longer the grain flling period, the more oil is produced. But if the grain flling period is short (for example, the grain is subject to drought stress during the flling period), due to the earlier formation of proteins, the grains will have a higher protein percentage. Moreover, due to the higher environmental temperature and water stress during the shorter grain flling stage, the seeds contain less fat. It is because under such conditions the ability to convert carbohydrates to fat is reduced and the oxidation of some unsaturated fatty acids reduces seed oil content (Farooq et al. [2017](#page-7-25); Asghari et al. [2018](#page-7-26)).

The decrease in lipids, under drought stress, can also be the result of reduction in: (1) seed size, (2) activity of lipid-synthesizing enzymes, (3) the amount of carbohydrates available for the storage tissues, and (4) lipase activity (Wang et al. [2019](#page-8-19); Li et al. [2020b](#page-7-27)). Mertz-Henning et al. [\(2018\)](#page-7-28) found a negative relationship between seed oil and protein under drought stress conditions. Table [2](#page-4-0) also shows that the treatments with the highest protein content have the least fat content and vice versa.

Treatment $D2 +$ macro resulted in the highest percentage of grain tannin, probably due to the presence of P and N afecting the production of plant phenolic compounds (Jiang et al. [2020](#page-7-29)). These results were in line with the results of Alhaithloul ([2019](#page-7-30)) indicating the increased concentration of tannins in *Artemisia sieberi* in dry conditions (dehydration). According to Jyothi and Hebsur [\(2017\)](#page-7-31), nano-fertilizer increases the total amount of phenolic and antioxidant compounds by afecting the chemical properties of cereals.

The least amount of seed tannin was related to D₂+ micro, which also resulted in the highest percentage of seed protein. This could be because when seed protein is present in large quantities, a relatively hydrophobic layer between the protein-tannin bond prevents protein from sedimentation. There were not signifcant diferences among diferent treatments, indicating that the percentage of sorghum seed tannin, when seed is exposed to drought stress during flling stage, is mostly afected by dehydration (Kaur and Asthir [2017;](#page-7-32) Alhaithloul et al. [2020](#page-7-33)).

Due to water stress at diferent stages of plant growth, nutrient uptake process is disrupted in diferent plant tissues. According to the results, under normal and non-stress irrigation conditions, the fertilizer application treatments similarly affected sorghum yield and biochemical properties. The treatments difer only in seed tannin and seed fat percentage. Accordingly, a combination of micro- and macro-nano-fertilizer can be applied in non-stress conditions to increase seed yields. In addition, the combination of micro+macro-fertilizer contained higher nutrients than chemical fertilizer (N, P, K). However, the tested nano-fertilizer enhanced sorghum yield and biochemical properties in drought stress conditions. Grain yield, Ca, and crude fat were most afected by nano- and chemical fertilizer in D1, and P, protein and tannin were most afected by nano- and chemical fertilizer in D2. The results indicate that the tested micro- and macro-fertilizer can favorably improve sorghum growth and biochemical properties in drought stress conditions. However, drought stress before and after fowering, and other environmental conditions determine the recommendation of superior fertilizer treatment between nano- and chemical fertilizer.

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Declarations

Conflict of interest The authors declare they do not have any confict of interest.

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