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Micronutrient Content and Geometrical Features of Grain Sorghum Subjected to Water Stress †

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Abstract: *Sorghum bicolor* L. Moench is a cereal producing reasonable yield in dry and semi-dry, hot and saline regions, where the production of other crops is limited. Sorghum grain is a rich source of minerals including magnesium (Mg), iron (Fe), zinc (Zn), Manganese (Mn), and calcium (Ca). However, the quantity of minerals can be dependent on the different agricultural and climatic conditions. Therefore, the objective of this study was to investigate the effect of water stress on the micronutrients (Ca, Fe, Mg, Mn, and Zn) and geometrical features of ten sorghum genotypes. There were different patterns observed in terms of grain micronutrients produced under normal and deficit irrigation depending on the genotype. A high concentration of Ca (878.9 ppm), Fe (335.8 ppm), and Mn (22.8 ppm) under normal irrigation and a value of Mg (1435.36–1783.21 and 1410.81–1890.95 ppm) and Zn (1.65–20.34 and 4.04–15.13 ppm) in normal and water stress conditions, respectively, was observed in the genotypes. Water stress had more influence on Ca, Fe, and Zn concentrations in comparison to Mg and Mn. The discrimination models based on the selected geometrical features of the sorghum grains produced under normal and deficit irrigation indicated a relatively low classification accuracy (40–67%). Therefore, an integrated approach using geometric and textural features is suggested to improve classification accuracy.

Keywords: drought; minerals; seed size; image processing; seed shape

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1. Introduction

Sorghum bicolor L. Moench is the fifth cereal worldwide that can produce reasonable yield under unfavorable environmental conditions, e.g., in dry and semi-dry, hot and saline regions, where the cultivation of other crops is not economical [1]. The production of crops has been adversely affected by environmental stresses. Water scarcity is one of the most limiting abiotic stresses that can be resulted in a reduction in crop yield in arid and semiarid areas [2].

Sorghum grain is a rich source of vitamins, protein, carbohydrates, energy, and minerals containing 1.30% to 3.30% of ash and minerals, e.g., magnesium (Mg), phosphorus, and potassium. Moreover, it is a better source of iron (Fe) and zinc (Zn) compared to rice and wheat concerning mineral contents [3]. Variation in the level of minerals in grains is influenced by genotype, environmental conditions, and interactions between the genotype and environmental conditions [4]. A maternal abiotic environment can influence offspring seed phenotype. Environmental factors such as water availability, temperature, salinity, fertilizer, and day length can potentially affect offspring seed production and traits [5].

While seed weight has often been considered as a key component of seed size, measures associated with seed size have not been used consistently in the literature. Seed size is a complex trait playing an important role in the adaptation of flowering crops to different environmental conditions. Larger seeds store more seed resources that enable seedlings to grow more rapidly and vigorously in the field [6]. Thus, screening of sorghum seeds produced under well-watered conditions is essential before processing.

Image analysis can be employed for seed classification and identification based on various morphological and textural features [7]. It has mainly been applied to classify cereal species or varieties; to identify poorly developed, shriveled, discolored, and insect-damaged kernels; to detect non-grain particles and non-cereal grains; and to estimate the ergosterol concentration of grain based on the thermal properties [8]. Therefore, the present investigation was performed to identify the effect of water stress on mineral contents (Ca, Fe, Mg, Mn, and Zn) and geometrical features (linear dimensions and shape factors) of 10 sorghum seeds. In addition, the possibility of these geometrical features in discrimination between seeds produced under normal and water deficit irrigation was evaluated.

2. Materials and Methods

2.1. Experimental Design

Sorghum genotypes, including two promising lines and eight accessions kindly provided by the National Plant Gene-Bank of Iran, were cultivated at the experimental field of Fars Agricultural and Natural Resources Research and Education Center on 6 June 2018 (52°43′ E and 29°46′ N; altitude 1604 m). The irrigation was considered as the main factor and the genotype as the sub-factor. Subplots were four rows 5 m long and a row distance of 0.6 m (12 m^2). The fertilizers were distributed according to the soil test. Irrigation was applied at three levels: normal irrigation (irrigation when the evaporation rates from pan class A reached 60 mm (Ir_{60})), mild deficit irrigation (irrigation when evaporation rates from pan class A exceeded 120 mm (Ir_{120})), and severe deficit irrigation (irrigation when evaporation rates from pan class A exceeded 180 mm (Ir_{180})). Deficit irrigation was applied from 26 days after sowing until the end of the season. The reference crop evapotranspiration (ET_o) was calculated using FAO-CROPWAT 8.0 to schedule different levels of irrigation. Grains were harvested at physiological maturity.

2.2. Mineral Content in Sorghum Grains

The grains produced under normal irrigation, and severe water stress were used for chemical analyses. Grains were washed for 15 s with deionized water and were dried with a paper towel. Then, the grain samples of each genotype were dried in an oven at 80 °C for 72 h. After drying, the grains of each genotype were finely grounded in the MF10 IKA mill (Staufen, Germany). The sorghum flour samples (0.100 g) were digested in 2 mL of HNO_3. After 24 h, the tubes were placed in a beaker containing water and heated on a hot block at 150 °C until complete digestion. At this point, the digested samples were diluted to 15 mL by adding double deionized water. Concentrations of Ca, Fe, Mg, Mn, and Zn were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, SPECTRO ARCOS, Kleve, Germany) in sorghum flour samples. The mineral concentrations were reported as ppm.

2.3. Image Analysis

The obtained images of sorghum grains were analyzed using the Mazda application (Łódź University of Technology, Institute of Electronics, Łódź, Poland). For each individual sorghum kernel, the region of interest (ROI) including one whole kernel was determined. In the case of each kernel with overlaid ROI, the geometric features including linear dimensions and shape factors were calculated. The geometric parameters were determined for one hundred kernels for each sorghum genotype and irrigation level.

2.4. Statistical Analysis

The differences in mean values of the mineral contents subjected to the different irrigation levels were determined using STATISTICA (StatSoft Inc., Tulsa, OK, USA) software at $p \leq 0.05$ using the Newman–Keuls test. The discriminant analysis was carried out using WEKA (Machine Learning Group, University of Waikato) software. The geometric parameters with the highest discriminative power were selected with the use of the Best First with the CFS (correlation-based feature selection) subset evaluator. For the discrimination of grain subjected to the different irrigation levels, the discriminative classifier of Logistic from group of Functions and test mode of 10-fold cross-validation were used. The applied feature selection method, classifier, and test mode ensured the highest classification accuracy. The confusion matrices and total accuracies of discrimination of sorghum grain subjected to different levels of irrigation were determined.

3. Results

The Ca content (ppm) of genotypes KGS23, TN-04-79, and TN-04-90 did not statistically change under normal and deficit irrigation (Table 1). The Ca value under deficit irrigation reduced approximately 52%, 26%, 72%, and 20% for genotypes MGS2, TN-04-78, TN-04-129, and TN-04-134, respectively, whereas the value ascended under deficit irrigation for TN-04-142, TN-04-59, and TN-04-86 around 41%, 53%, and 17%, respectively (Table 1). The means of grain Fe tended to be statistically equal under normal and deficit irrigation for genotypes MGS2, TN-04-129, and TN-04-134. Grain Fe content tended to be higher in deficit irrigation for genotypes KGS23, TN-04-78, TN-04-59, and TN-04-86, whereas a reduction in grain Fe was observed in deficit irrigation for TN-04-79, TN-04-142, and TN-04-90. The highest increase and reduction in Fe concentration under deficit irrigation occurred at 234% and 47%, respectively (Table 1).

Table 1. Mean values of micronutrients of sorghum grain subjected to normal irrigation and severe deficit irrigation.

Genotype, Irrigation	Parameter				
	Ca (ppm)	**Fe (ppm)**	**Mg (ppm)**	**Mn (ppm)**	**Zn (ppm)**
MGS2, Ir_{60}	878.94 ^{a}	335.81 ^{a}	1783.21 ^{a}	22.76 ^{a}	14.73 ^{a}
MGS2, Ir_{180}	425.55 ^{b}	255.91 ^{a}	1736.36 ^{a}	22.30 ^{a}	14.50 ^{a}
KGS23, Ir_{60}	295.83 ^{a}	111.82 ^{a}	1695.43 ^{a}	14.66 ^{a}	8.71 ^{a}
KGS23, Ir_{180}	315.95 ^{a}	373.22 ^{b}	1890.95 ^{a}	17.96 ^{a}	14.91 ^{b}
TN-04-78, Ir_{60}	522.61 ^{a}	84.20 ^{a}	1554.29 ^{a}	12.12 ^{a}	4.82 ^{a}
TN-04-78, Ir_{180}	385.63 ^{b}	208.35 ^{b}	1618.30 ^{a}	23.79 ^{b}	15.13 ^{b}
TN-04-79, Ir_{60}	591.94 ^{a}	191.80 ^{a}	1681.53 ^{a}	20.04 ^{a}	20.34 ^{a}
TN-04-79, Ir_{180}	597.65 ^{a}	100.82 ^{b}	1661.53 ^{a}	14.84 ^{b}	13.97 ^{b}
TN-04-129, Ir_{60}	578.03 ^{a}	49.96 ^{a}	1496.56 ^{a}	10.47 ^{a}	3.15 ^{a}
TN-04-129, Ir_{180}	153.44 ^{b}	106.26 ^{a}	1410.81 ^{a}	13.44 ^{a}	6.91 ^{b}
TN-04-134, Ir_{60}	558.88 ^{a}	106.13 ^{a}	1528.70 ^{a}	12.08 ^{a}	1.65 ^{a}
TN-04-134, Ir_{180}	447.33 ^{b}	159.16 ^{a}	1579.99 ^{a}	14.95 ^{b}	5.02 ^{b}
TN-04-142, Ir_{60}	287.00 ^{a}	166.88 ^{a}	1435.36 ^{a}	5.68 ^{a}	6.96 ^{a}
TN-04-142, Ir_{180}	405.39 ^{b}	112.26 ^{b}	1874.30 ^{b}	10.39 ^{b}	14.92 ^{b}
TN-04-59, Ir_{60}	502.82 ^{a}	35.26 ^{a}	1510.95 ^{a}	11.82 ^{a}	11.11 ^{a}
TN-04-59, Ir_{180}	771.29 ^{b}	71.34 ^{b}	1646.83 ^{a}	13.41 ^{a}	4.04 ^{b}
TN-04-86, Ir_{60}	580.96 ^{a}	95.72 ^{a}	1606.58 ^{a}	19.09 ^{a}	7.33 ^{a}
TN-04-86, Ir_{180}	680.00 ^{b}	266.97 ^{b}	1734.27 ^{a}	28.15 ^{b}	9.28 ^{a}
TN-04-90, Ir_{60}	501.32 ^{a}	135.35 ^{a}	1769.99 ^{a}	14.57 ^{a}	6.38 ^{a}
TN-04-90, Ir_{180}	613.56 ^{a}	74.12 ^{b}	1778.01 ^{a}	14.51 ^{a}	6.75 ^{a}

Ir_{60}—normal irrigation; Ir_{180}—severe deficit irrigation; ^{a,b}—the same letters in columns denote no statistical differences between samples for one genotype.

A mean comparison of Mg content revealed no significant difference between normal and deficit irrigation except for genotype TN-04-142 (30% higher Mg content under deficit irrigation). There were different patterns observed in the term of grain Mn content. The values of Mn suggested that the genotypes MGS2, KGS23, TN-04-129, TN-04-59, and TN-04-90 were not statistically different in the term of Mn concentration under normal and deficit irrigation. While there was a decrease of 26% in Mn content for the grains of TN-04-79 under deficit irrigation, an increase in Mn concentration equal to 96%, 24%, 74%, and 47% was observed for genotypes TN-04-78, TN-04-134, TN-04-142, and TN-04-86, respectively (Table 1).

Geometrical features including linear dimensional and shape factors were calculated to develop discriminative models for the classification of 10 sorghum genotypes grown under different levels of irrigation. The discriminative models reached an accuracy of 40% to 68% (Table 2). It is not a satisfactory result indicating that 120 out of 300 cases to 204 out of 300 cases were correctly classified. The highest and slightest distinction between grains produced under different irrigation regimes occurred for KGS23 and TN-04-79, respectively. It corroborates that the effect of water deficit irrigation on the grain geometric features of sorghum grains is not definite, and there is a high similarity between the grains produced under normal and deficit irrigation.

Table 2. The confusion matrices and accuracies of discrimination of sorghum grain subjected to normal irrigation, mild deficit irrigation, and severe deficit irrigation based on a set of selected geometric features.

Classifier	Predicted Class (%)			Average Accuracy (%)	Predicted Class (%)			Average Accuracy (%)	Actual Class
	Ir_{60}	Ir_{120}	Ir_{180}		Ir_{60}	Ir_{120}	Ir_{180}		
	MGS2				**TN-04-134**				
functions.Logistic	53	34	13	54	48	34	18	47	Ir_{60}
	27	43	30		21	43	36		Ir_{120}
	11	23	66		12	37	51		Ir_{180}
	KGS23				**TN-04-142**				
functions.Logistic	14	23	63	40	50	30	20	48	Ir_{60}
	4	52	44		38	23	39		Ir_{120}
	12	34	54		14	16	70		Ir_{180}
	TN-04-78				**TN-04-59**				
functions.Logistic	33	61	6	43	62	26	12	59	Ir_{60}
	12	69	19		28	55	17		Ir_{120}
	9	65	26		18	23	59		Ir_{180}
	TN-04-79				**TN-04-86**				
functions.Logistic	54	42	4	68	43	22	35	46	Ir_{60}
	39	57	4		22	45	33		Ir_{120}
	2	6	92		30	19	51		Ir_{180}
	TN-04-129				**TN-04-90**				
functions.Logistic	53	35	12	48	73	24	3	58	Ir_{60}
	20	72	8		28	65	7		Ir_{120}
	31	49	20		28	36	36		Ir_{180}

Ir_{60}—normal irrigation; Ir_{120}—mild deficit irrigation; Ir_{180}—severe deficit irrigation.

4. Discussion

We found a much higher concentration of Ca (878.9 ppm), Fe (335.8 ppm), and Mn (22.8 ppm) under normal irrigation than those quantified by Martino et al. (2012) as follows: Ca (0.06–0.19 g/kg), Fe (4.7–14.9 mg/kg), Mg (0.79–1.47 g/kg), Mn (not detected-0.6 mg/kg), and Zn content (13.2–27.0 mg/kg). A wide variation for concentrations of Fe (1.10–9.54 mg 100 g^{-1}) and Zn (1.12–7.58 mg 100 g^{-1}) in 112 local landraces and varieties of sorghum was observed [4]. On the other hand, the Mg and Zn contents of our study were to some extent in agreement with those found by [4] who found Mg (1.28–2.43 and 0.88–1.84 g/kg) and Zn (16.21–45.78 and 12.81–38.98 mg/kg) in the normal and water stress condition [9].

The difference in results between these studies might be due to genotype, soil, and climate conditions that influence mineral concentrations. A smaller content of Ca (0.15 g/kg) was reported, but there were nearly similar concentrations of Mg (1.64 g/kg), Mn (20.6 mg/kg) and Zn (29.7 mg/kg) content in sorghum genotypes from Africa [10]. The Fe content of sorghum genotypes reported by [11] (58 mg/kg) and [12] (53.4 mg/kg) also was lower than the contents found in the present study. As presented in Table 2, water stress had more influence on Ca, Fe, and Zn concentrations followed by Mn and the Mg content influenced the least by water deficit irrigation. The results by [4] indicated that only 30%, 45%, and 51% of the genotypes showed a significant difference in terms of Ca, Fe, and Zn between normal and water stress.

In the literature, the discrimination of sorghum grains subjected to water stress has never been evaluated based on image processing and geometric parameters (linear dimensions and shape factors). The discrimination of healthy and Fusarium-infected wheat kernels using geometric parameters was not useful [2]. However, the incorporation of color parameters R (Red), G (Green), and B (Blue) from RGB color space and descriptor H (Hue) from HSL color space into the discrimination model increased accuracy to 85%.

Classification accuracies of wheat kernels as healthy, chalky, and shriveled with 68.4% and 56.9% for kernels infected by Fusarium culmorum and Fusarium graminearum, respectively, were obtained [13]. Wheat kernels infected by Fusarium were classified based on morphological features with accuracies ranging from 58.12% to 73.37% [14]. Therefore, other features, e.g., texture should also be included in the discrimination model to increase classification accuracy.

5. Conclusions

There was a high genetic variability for mineral content between the genotypes and water regimes. Regarding the micronutrient elements of grains, the concentration of Ca and Fe in the genotypes was much higher than those found by other authors. Thus, they can be highlighted as potential sources of Ca and Fe in sorghum breeding and biofortification programs. In addition, it may be noted that genotype, soil, and climate are factors that may influence the mineral concentration. Grain geometric features demonstrated to be an inappropriate method for discrimination of sorghum grains produced under normal irrigation and water deficit irrigation. Classification accuracy ranged from 40% to 67% based on the selected linear dimensions and shape factors incorporated into the model. Therefore, an integrated approach using geometric and textural features can improve classification accuracy. Furthermore, the effect of water stress on the linear dimensions and shape factors was genotype-dependent.

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