



Grain yield and micronutrient concentrations of maize parental lines of new hybrid genotypes affected by the foliar application of micronutrients

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Abstract It is important for the breeders to find how genetic differences may affect crop grain yield and nutrient uptake affected by micronutrient fertilization. Accordingly, with respect to our published research, the foliar application of the most deficient micronutrients (Fe, Zn and Mn) in the arid and semi-arid areas of the world affecting grain yield and nutrient concentration of maize parental lines of new hybrid genotypes was tested in a two-year experiment (2016–2017). A split plot experiment (randomized complete block design) with seven maize parental lines (G1–G7, sub-plots), and eight micronutrients treatments (main plots) including control (without spraying, M1), Zn (M2), Mn (M3), and Fe (M4) at 3 g L^{-1} , Mn + Zn (M5), Fe + Zn (M6), Fe + Mn (M7), and Fe + Mn + Zn (M8) at 1.5 g L^{-1} , sprayed at the growth stages of V8 and the full appearance of the plant organs (R1) was conducted. Plant height, cob height, 1000 grain weight, grain yield, number of rows per cob, number of grains per row, grain crude protein content, and micronutrient (Zn, Fe and Mn) concentrations were determined. Micronutrients significantly affected Fe ($27.68\text{--}62.55 \text{ mg. kg}^{-1}$) and Zn ($33.34\text{--}55.73 \text{ mg. kg}^{-1}$) concentrations. A3 ($12,600 \text{ kg. ha}^{-1}$) and A5 (8900 kg. ha^{-1}) resulted in the highest and least grain yield, respectively. M7 ($11,470 \text{ kg. ha}^{-1}$) had the highest grain yield significantly different from control (5510 kg. ha^{-1}). Interestingly, just Mn significantly affected grain crude protein ($9.63\text{--}12.92\%$). Correlation

coefficients indicated Mn and Fe as the least and the most correlated micronutrients with the growth of maize parental lines.

Keywords Crop breeding · Hybrid lines · Grain protein · Grain weight, iron · Leaf area · Manganese · Zinc

Introduction

Maize, which is mainly cultivated for grain yield, is a highly desired forage for livestock, and is also unique in terms of energy supply, for chicken and subsequent egg production compared to other cereals. Approximately, 20–25% of the world's maize production is turned into flour, starch, pastry, canning, porridge, oil and syrup for human foods, 60–75% into grain, pulp, and powder to feed livestock, and about 5% for industrial purposes such as alcohol production (Martinez and Fernandez 2019; Rausch et al. 2019). More than 500 types of second grade products are produced using maize, and maize stems are used in the paper and cardboard industry (Klopfenstein et al. 2013; Aguiar et al. 2021).

Due to the process of global warming and the deficiency of water, maize plants, in the arid and semi-arid areas have been subjected to yield reduction, and the use of suitable practice, which may alleviate such stressful conditions, is unavoidable (Wang et al. 2021). Due to the imbalance use of chemical fertilizers and the improper handling of agricultural lands after harvesting, excess and deficiency of nutrients may reduce maize yield and result in the pollution of the environment (Miransari and Mackenzie 2014, 2015). High pH and calcium carbonate content in some parts of the arid and semi-arid areas of the world including Iran, make micronutrients unavailable for maize

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uptake and subsequently decrease maize grain yield and quality (Miransari 2013; Zhang et al. 2017a; Hadebe et al. 2021). Micronutrients are essential for human health; however, their concentration is not high in maize (Zhang et al. 2017b; Sharma et al. 2021). Accordingly, using a suitable method to provide maize with the required micronutrients including iron (Fe), zinc (Zn) and manganese (Mn), at the right time, is of outmost significance. Micronutrients are important for crop yield and grain quality, due to their important functioning in plant by: (1) affecting carbohydrate metabolism, (2) preventing nitrate accumulation, by influencing nitrogen metabolism, activating oxidation reactions, and reducing and transferring electrons, and (3) stimulating photosynthetic activity, which increases the content of total soluble solids (Rasool et al. 2019; Sabet and Mortazaeinezhad 2018; Asadi et al. 2019).

The excess use of fertilizers, especially phosphate fertilizers, reduces Zn solubility, due to the production of insoluble compounds (Gao and Ma 2015; Maqbool and Beshir 2019; Bagheri et al. 2021). High bicarbonate concentration in irrigation water increases cellular bicarbonate absorption and cellular sap pH, resulting in Zn deposition in the vessels (Zhang et al. 2017b; 2017c).

The three micronutrients of Fe, Zn and Mn are among the most important micronutrients (Subedi, and Ma, 2009; Ma and Zheng, 2018) affecting plant growth and yield production. Fe plays an important role in the cytochrome structure as an electron carrier in photosynthetic systems for the processes of respiration, oxidation, reduction, and production of chlorophyll. Zn is required for the activity of dehydrogenase and proteinase enzymes, formation of RNA and growth regulation. The sterility of pollen grains, small leaf size, the presence of light strips along the main leaf vein and plant dwarf are symptoms of Zn deficiency. Mn plays a role in the synthesis of respiratory and photosynthetic enzymes, and prevents nitrate accumulation in the plant tissues. Reducing plant growth and height, yellowing, sterility of pollen grains and reducing tiller number in plant are complications of manganese deficiency (Römheld and Marschner 1991; Saha et al. 2019; Singh and Dwivedi 2019).

The other important parameter, which determines plant response to micronutrient deficiency is the plant's genetic structure. Different plant species may respond differently to micronutrient deficiency (Ahmadzadeh Chaleshtori et al., 2020). Accordingly, breeders select the tolerant species and cross them with the non-tolerant ones to improve the tolerance of the latter. Cropping plants, which are resistant under micronutrient deficiency is one of the suitable and efficient methods to prevent the reduction of yield and quality (Marschner 2011; Woli et al. 2019; Pramitha et al. 2020).

With respect to the above-mentioned details, finding the most tolerant maize species under micronutrient deficiency in combination with an effective, economic and environmentally friendly method to alleviate micronutrient deficiency is an important research aspect. Although there has been previous research on the use of micronutrient spraying and resistant crop plants under micronutrient deficiency, more has yet to be investigated on the alleviation of micronutrient efficiency by spraying and use of resistant genotypes. According to our published research (Khalafi et al., 2021), the objective was to investigate the effects of the foliar application of the most deficient micronutrients (Fe, Zn and Mn) in the arid and semi-arid areas and different parental lines of maize new hybrid genotypes on grain yield and micronutrient concentrations in a two-year field experiment in the northern part of Khuzestan province, Iran.

Materials and methods

Experimental sites

The experiments were conducted in 2016 and 2017 in the Research Station of Safiabad Dezful, Khuzestan province, Iran, in the eastern longitude of 48° 32' and northern latitude of 32° 22', with the altitude of 82 m. The climate is warm and dry during the summer with humid and rainy winter. The average temperature (a region with warm climate) is in the range of 31.2–52 °C during the summer, and from below 0 to 14.9 °C in the winter, with a mean annual total precipitation of 265 mm.

Experimental treatments

The experiment was a split plot on the basis of a randomized complete block design with seven parental lines of maize new hybrid genotypes (G1-G7), devoted to the sub plots, and eight micronutrients treatments including control (without spraying, M1), Zn (M2), Mn (M3), and Fe (M4) at 3 g L⁻¹, Mn + Zn (M5), Fe + Zn (M6), Fe + Mn (M7), and Fe + Mn + Zn (M8) at 1.5 g L⁻¹, tested in the main plots (a total number of 224 experimental plots, 7 × 8 × 4). Accordingly, the single treatments contained the nutrient at 3 g L⁻¹, and the combined treatments contained each nutrient at 1.5 g L⁻¹. The selected lines for investigation, are the parental lines of three commercial maize hybrids selected at the final stage of self-pollination (sixth generation, F6), for drought tolerance. Accordingly, the selected genotypes are the parental lines of new hybrids, which have been genetically modified for higher yield production in the arid and semi-arid areas of the world including Iran.

The micronutrient solutions, developed on the basis of corn nutritional requirements in the arid and semi-arid areas of the world, were prepared by dissolving 60 g of each micronutrient chelate (collected from the Iranian vendors) in 20 L of water (Khalafi et al. 2021). The foliar spray was conducted at two different growth stages including V8 and the full appearance of the plant organs (R1). It is because at the V8 stage maize may have the highest rate of nutrient uptake, and at the full appearance of the reproductive organs will also absorb nutrients. Water ($EC = 0.635 \text{ dS. m}^{-1}$, $pH = 7.62$, total dissolved solid = 400 meq. L^{-1} , sodium adsorption ratio = 1.64 meq. L^{-1}) and soil chemical properties of the experimental field were determined using the standard methods (Miransari et al. 2008) (Table 1). For the measurement of soil physicochemical properties, ten soil samples were randomly collected from the depth of 0–30 cm, and were thoroughly mixed and then one composite sample was used for the analyses.

Agronomical practices

The field was prepared by first irrigating the field, and then cultivating at the suitable moisture. The field was disked three times and leveled. The plots measured $3 \times 6 \text{ m}$ (total area of the field 2500 m^2) with 7 rows (75 cm spacing) of planted maize at 75,000–80,000 plants per hectare. The fields in the two years were surface irrigated daily for seven days. The field was fertilized before seeding with ammonium phosphate at 125 kg. ha^{-1} , potassium sulfate at 140 kg. ha^{-1} and urea at 250 kg. ha^{-1} according to the recommendation in the regions. The weeds were removed by hand, and the agronomical practices were according to the farmers in the region.

Measurements

Five plant samples were taken at the physiological maturity from the center of each plot (two rows) with a length of six meters. Different maize growth and grain yield components including plant height (H), cob height (Hc), leaf area (LA), number of grains rows (NR), number of grains per rows (NG), 1000-grain weight (W), grain yield (Y), grain protein content (Pr), and grain micronutrient concentration were determined. Micronutrients were measured by the method of Katyal and Sharma (1980) and Sparks et al. (2020) using o-phenanthroline (o-Ph) and spectrophotometer according

to the following details. 1–10 o-phenanthroline (o-Ph) was used as a stable extractant and efficient chelator for the nutrients of the plant samples resulting in the development of a chelate complex with an orange color, which was subsequently read by spectrophotometer (Katyal and Sharma 1980).

Statistical analysis

Data were subjected to analysis of variance using SAS Proc GLM. Means were compared using Proc means (least significant difference, LSD at $P \leq 0.05$). The correlations among different measured traits were determined using Proc Corr. The single and the interaction effects of data were plotted using Proc Plot.

Results

Analysis of variance indicated the single and the interactions of the experimental treatments significantly affected plant growth and yield components (Table 2).

Maize growth

Plant height (H)

Plant H was significantly higher in 2016 (155 cm) than that in 2017 (148 cm). Genotypes differed significantly in plant heights, with genotype 7 (188 cm) being the highest and genotype 5 (121 cm) being the lowest. Treatment M8 resulted in significantly taller plants (161 cm) than M3 (153 cm) and the control (117 cm) (Table 3). The interactions of year and genotypes and year and micronutrients on H are presented in Figs. 1 and 2, respectively.

Cob height (Hc)

The mean of Hc was significantly higher in 2017 (61.85 cm) than in 2016 (60.15 cm). Genotypes 4 (70.31 cm) and 5 (50.95 cm) resulted in the highest and least Hc, respectively, significantly different from the other genotypes. The highest Hc resulted in M5 (63.79 cm) significantly different from M2 (61.52 cm) and control (47.30 cm) (Table 3). The interactions of year and genotypes, and year and micronutrients on Hc have been presented in Figs. 1 and 2, respectively.

Table 1 Soil and water chemical properties

Sample	EC (dS. m^{-1})	pH	Organic carbon (%)	Mn	Cu	Fe (mg. kg^{-1})	Zn
Soil	4.01	7.50	1.00	4.8	1.4	6.00	1.2

Table 2 Analysis of variance indicating the significance of the single and interaction effects of the experimental treatments affecting the measured traits

S.V	d.f	Pr > F										
		H	Hc	LA	W	GY	NR	NG	Pr	Zn	Mn	Fe
Yr	1	< .0001	0.0008	0.0043	0.3022	< .0001	< .0001	0.0070	< .0001	< .0001	0.0429	0.0804
G	6	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
M	7	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
G*M	42	< .0001	0.0702	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
Yr*G	6	< .0001	0.9675	0.8417	1.0000	0.0049	0.1696	0.9035	0.3758	< .0001	0.7169	0.1940
Yr*M	7	0.9999	1.0000	0.9998	1.0000	0.9813	0.6812	0.8905	0.8790	< .0001	0.9959	0.0637
Yr*G*M	84	< .0001	0.9780	0.0002	< .0001	< .0001	0.0056	< .0001	0.1532	< .0001	0.0574	< .0001

F: F-test, Pr: probability, S.V.: source of variation, d.f. degree of freedom, Yr: year, G: genotype, M: micronutrient, H: maize height, Hc: cob height, LA: leaf area, W: weight of 1000 grains, GY: grain yield, NR: number of grain rows, NG: number of grains in a row, Pr: crude protein, Zn: zinc, Mn: manganese, Fe: iron

Table 3 The mean comparisons of the measured parameters affected by the experimental treatments, averaged for the 2 years

G	H cm	Hc cm	LA cm ²	W g	Y kg. ha ⁻¹	NR	NG	Pr %	Fe	Zn mg. kg ⁻¹	Mn
G1	148d	57.95d	25.28 g	189.02e	9080e	15.76b	25.54	11.08e	53.25b	47.70b	16.45a
G2	154c	63.06bc	28.33f	202.77d	10560d	15.41b	24.16b	11.75 cd	56.64b	51.64	14.41b
G3	152 cd	62.27c	33.58e	242.94a	12600a	13.52d	26.33a	11.45de	36.58d	44.52c	13.84bc
G4	166b	70.31a	35.72d	209.22c	11050c	11.18f	17.53e	11.99c	44.42c	34.98e	11.16d
G5	121f	50.95e	65.13b	176.66f	8900e	14.47c	20.14d	13.12a	44.17c	39.75d	12.25 cd
G6	132e	57.86d	60.09c	180.11f	9220e	17.78a	21.33c	12.79ab	47.97c	44.05c	14.86ab
G7	188a	64.25b	69.03a	231.02b	11660b	12.28e	26.58a	12.63b	67.94a	43.73c	12.17 cd
EMS	123.68	31.83	25.89	334.5	1370	2.22	11.19	1.43	177.09	40.82	32.43
LSD	3.87	1.96	1.77	6.36	410	0.52	1.16	0.42	4.63	2.22	1.98
M	H cm	Hc cm	LA cm ²	W g	Y kg. ha ⁻¹	NR	NG	Pr %	Fe	Zn mg. kg ⁻¹	Mn
M1	117e	47.30c	29.43b	103.96d	5508d	12.77e	19.07e	9.63c	27.68d	33.52d	8.25c
M2	155 cd	61.52b	48.61a	211.34c	10652c	14.12 cd	24.21ab	12.43b	41.64c	50.82b	15.71a
M3	153d	63.12ab	47.70a	213.57c	10880bc	13.9d	21.21d	12.92a	46.86b	33.34d	15.31a
M4	156bcd	63.68a	47.52a	215.50bc	10902bc	14.69ab	24.87a	12.30b	60.02a	37.73c	13.09b
M5	155 cd	63.79a	46.88a	222.34a	11430a	14.39bc	24.05ab	12.58ab	50.11b	51.09b	13.72ab
M6	158abc	62.55ab	47.30a	221.66ab	11309ab	15.02a	23.45bc	12.27b	62.55a	55.73a	13.80ab
M7	160ab	62.75ab	46.90a	223.80a	11471a	14.97a	22.55c	12.52ab	51.00b	38.80c	15.829a
M8	161a	62.91ab	48.13a	224.07a	11374a	15.18a	25.23a	12.25b	61.25a	49.11b	13.03b
EMS	123.68	31.83	25.89	334.5	1370	2.22	11.19	1.43	177.09	40.82	32.44
LSD	4.13	2.10	1.89	6.80	440	0.55	1.24	0.44	4.95	2.38	2.12

Yr: year, G: genotype, M: micronutrient, EMS: error mean square, LSD: least significant difference (alpha = 0.05), H (cm): maize height, Hc (cm): cob height, LA: leaf area index, W: weight of 1000 grains (g), Y (kg/ha): grain yield, NR: number of grain rows., NG: number of grains in a row, Pr: crude protein, Zn (mg/kg): zinc, Mn (mg/kg): manganese, Fe (mg/kg): iron. The error degree of freedom is 336 and the critical value of “t” is 1.97. M1(control), Zn (M2), Mn (M3), Fe (M4), Mn + Zn (M5), Fe + Zn (M6), Fe + Mn (M7), and Fe + Mn + Zn (M8)

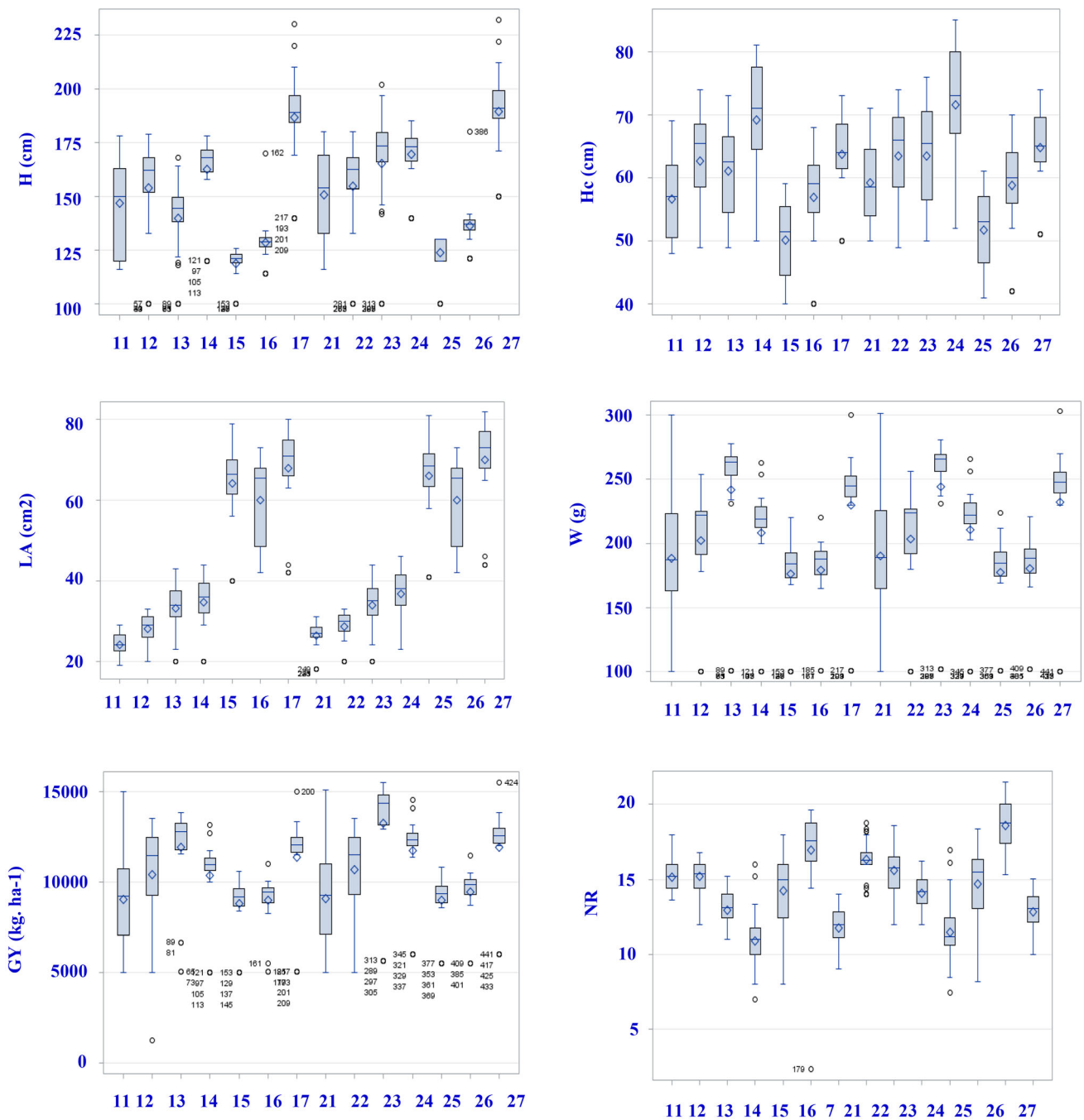


Fig. 1 The box plots indicating the interaction effects of the year (1 and 2) and genotype (1, 2, 3, 4, 5, 6, and 7) on the measured parameters. H: maize height, Hc: cob height, LA: leaf area, W: weight of 1000 grains, GY: grain yield, NR: number of grain rows. The box

plots indicating the interaction effects of year and genotype on the measured parameters. NG: number of grains in a row, Pr: crude protein, Zn: zinc, Mn: manganese, Fe: iron

Leaf area (LA)

Leaf area in 2016 (44.61 cm²) was significantly different from that of 2017 (46.00 cm²). Leaf area was the highest in genotype 7 (69.03 cm²) and the least in genotype 1 (25.28 cm²). There were no significant differences among the micronutrient treatments affecting LA, however,

significantly higher than the control treatment (Table 3). The interactions of year and genotypes, and year and micronutrients affecting LA have been presented in Figs. 1 and 2, respectively.

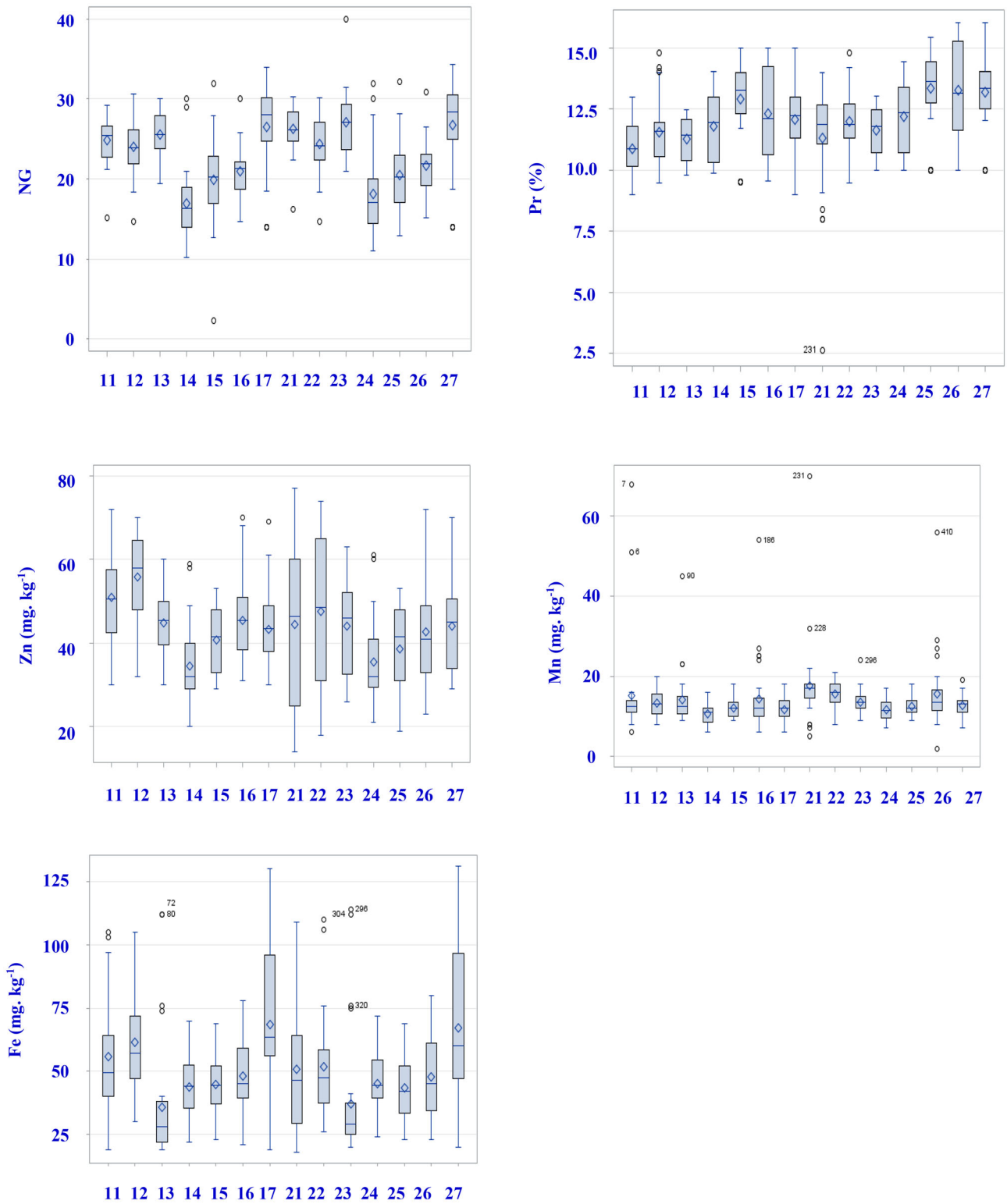


Fig. 1 continued

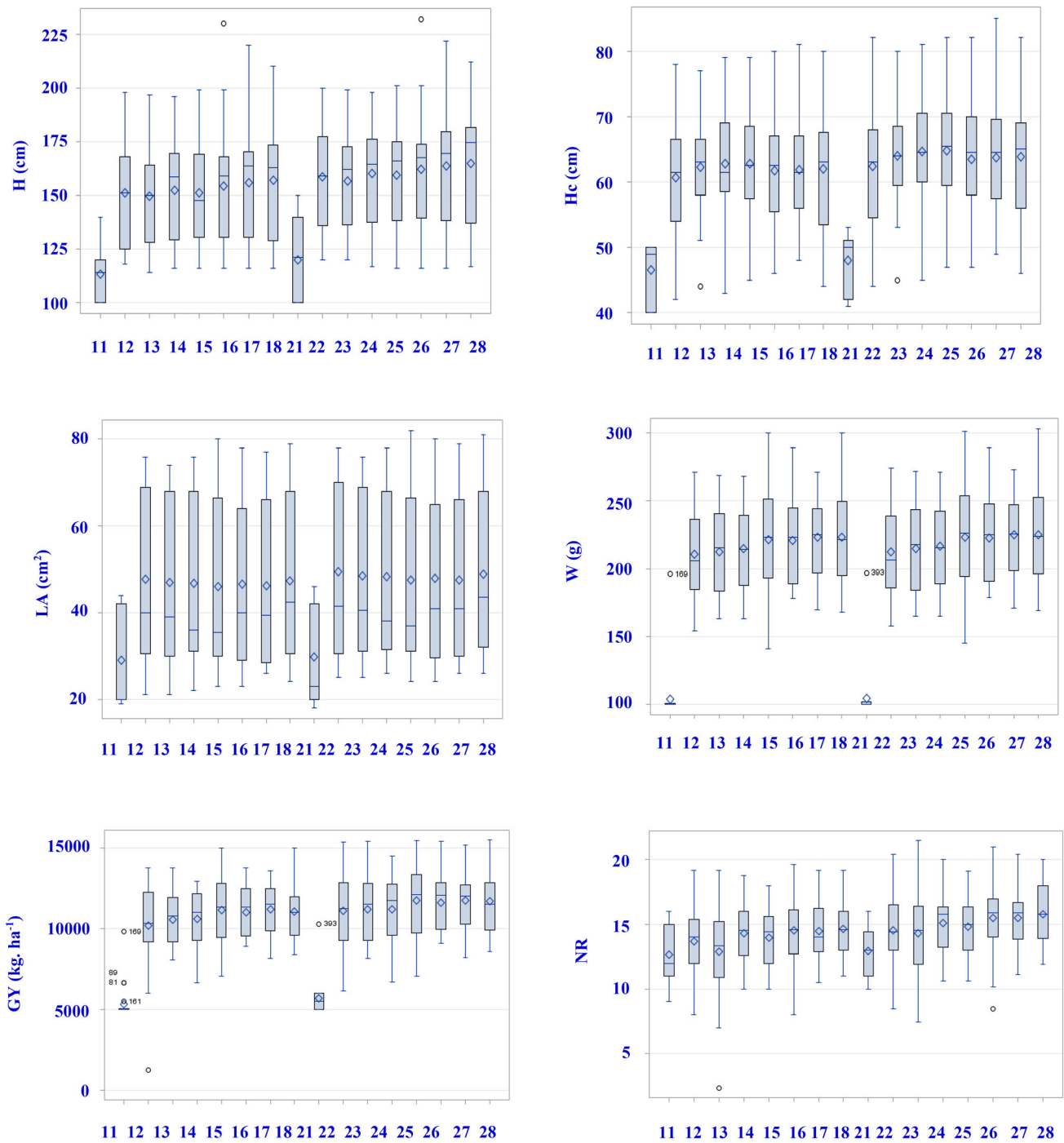


Fig. 2 The box plots indicating the interaction effects of year and micronutrients on the measured parameters. H: maize height, Hc: cob height, LA: leaf area, W: weight of 1000 grains, GY: grain yield, NR: number of grain rows. The box plots indicating the interaction effects

of year and micronutrients on the measured parameters. NG: number of grains in a row, Pr: crude protein, Zn: zinc, Mn: manganese, Fe: iron

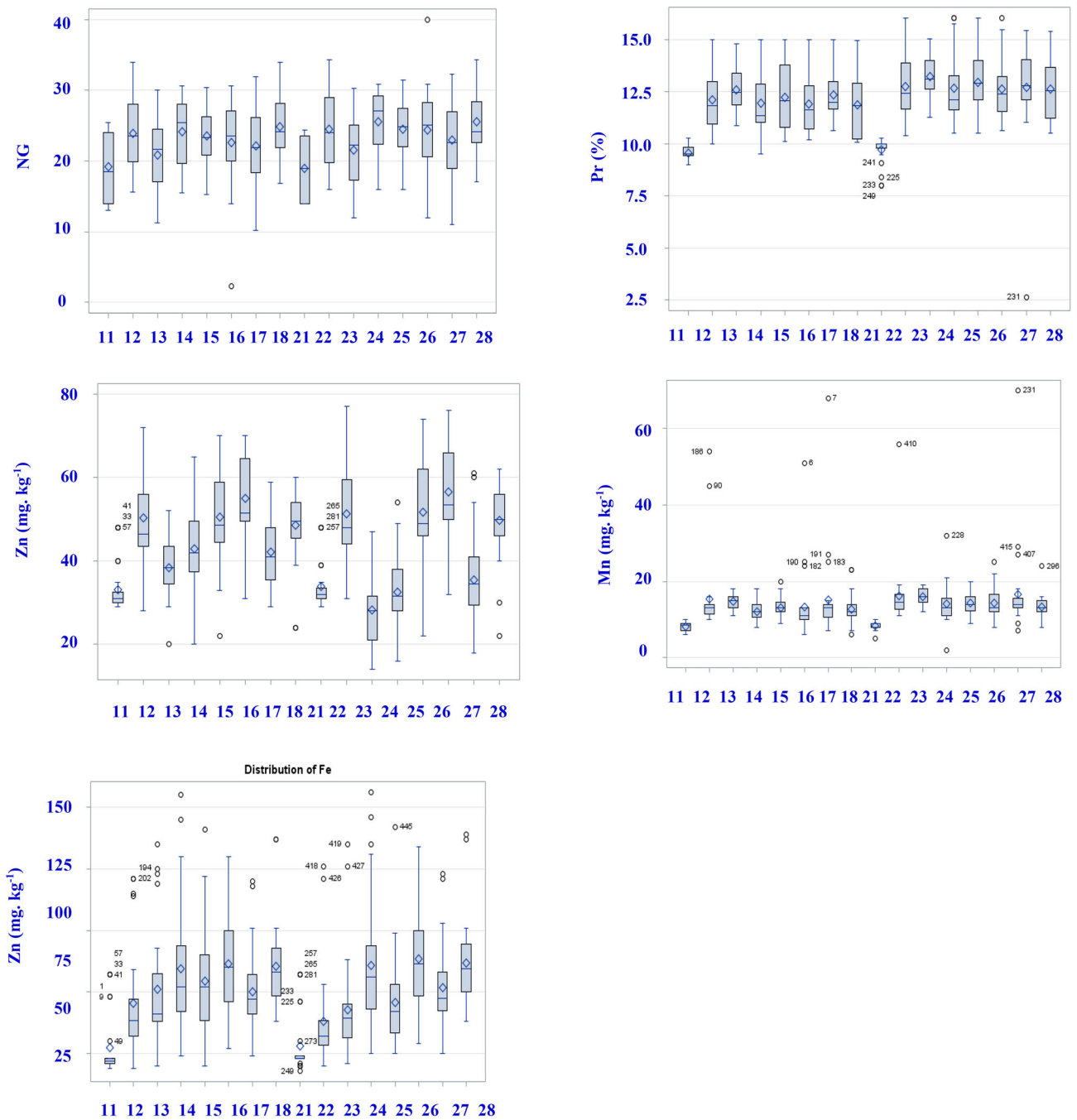


Fig. 2 continued

Yield and grain protein

Weight of 1000 grain (W)

There was not a significant difference between 2016 (203.64 g) and 2017 (205.42 g) in terms of W. Genotype 3 had the highest W (242.94 g) and genotyped 5 had the least W (176.66 g), significantly different from the other treatments. M8 resulted in the highest (224.07 g) W,

significantly different from the least one (M1, 103.97 g) (Table 3). The interactions of year and genotypes, and year and micronutrients affecting W have been presented in Figs. 1 and 2, respectively.

Grain yield (Y)

The year 2017 resulted in a significantly higher grain yield (10,074 kg. ha⁻¹) compared with that of 2016 (10,014 kg.

ha⁻¹). The highest and the least Y were related to genotype 3 (12,600 kg. ha⁻¹) and genotype 5 (8900 kg. ha⁻¹). The highest grain yield resulted in M7 (11,470 kg. ha⁻¹) significantly different from the control (5510 kg. ha⁻¹) (Table 3). The interactions of year and genotypes, and year and micronutrients affecting Y have been presented in Figs. 1 and 2, respectively.

Number of grain rows (NR)

There was a significantly higher NR in 2017 (14.80) than that in 2016 (13.88). Genotypes 6 (17.82) and 4 (11.18) had the highest and the least NR, respectively. M8 and M1 resulted in the highest (15.18) and the least NR (12.81), respectively (Table 3). The interactions of year and genotypes, and year and micronutrients on NR are presented in Figs. 1 and 2, respectively.

Number of grains in a row (NG)

NG was significantly higher in 2017 (23.51) compared with 2016 (22.66). The highest NG was resulted by genotype 7 (26.58), followed by genotype 3 (26.33) and genotype 1 (25.54) significantly different from the other treatments, including the least one (genotype 4, 17.53). M8 (25.23), M4 (24.87), M2 (24.21) and M5 (24.05) had the highest NG significantly higher than M3 (23.23) and control (19.07) (Table 3). The interactions of year and genotypes, and year and micronutrients on NG are presented in Figs. 1 and 2, respectively.

Grain crude protein (Pr)

Grain crude protein was significantly higher in 2017 (12.41) than in 2016 (11.82%). The highest Pr was related to genotype 5 (13.12) followed by genotype 6 (12.79) and genotype 7 (12.63%) significantly different from the other genotypes. The highest Pr was resulted by M3 (12.92), M5 (12.59) and M7 (12.52) significantly higher than the control (9.63%) (Table 3). The interactions of year and genotypes, and year and micronutrients affecting Pr are presented in Figs. 1 and 2, respectively.

Nutrient uptake

Zn concentration

Maize grain Zn in 2016 (45.11 mg. kg⁻¹) was significantly higher than in 2017 (42.42 mg. kg⁻¹). Genotypes 2 (51.64) and 4 (35.00 mg. kg⁻¹) resulted in the highest and the least Zn concentration. Treatment M6 (55.73 mg. kg⁻¹) was the one, which resulted in the highest Zn concentration with significant differences from the other treatments including

control (33.34 mg. kg⁻¹) (Table 3). The interactions of year and genotypes, and year and micronutrients affecting Zn are presented in Figs. 1 and 2, respectively.

Mn concentration

Maize grain Mn in 2017 (14.14 mg. kg⁻¹) was higher than in 2016 (13.04 mg. kg⁻¹). The highest and the least Mn concentration were related to genotype 1 (16.45) and genotype 4 (11.16 mg. kg⁻¹), respectively, significantly different from the other treatments. Maize grain Mn was not affected as much compared to Fe and Zn under the experimental treatments however there was not much differences among the treatments. However, M7 (15.83) and M1 (8.25 mg. kg⁻¹) resulted in the highest and the least Mn concentration, respectively (Table 3). The interactions of year and genotypes, and year and micronutrients affecting Mn are presented in Figs. 1 and 2, respectively.

Fe concentration

There was not a significant difference between 2016 (51.24 mg. kg⁻¹) and 2017 (49.04 mg. kg⁻¹) in terms of Fe concentration. Genotype 7 (67.94 mg. kg⁻¹) and 3 (36.58 mg. kg⁻¹) had the highest and the least Fe concentration, respectively, significantly different from the other treatments. M6 (62.55), M8 (61.25 mg. kg⁻¹) and M4 (60.00 mg. kg⁻¹) resulted in the highest Fe concentration significantly higher than the other treatments including control (27.68 mg. kg⁻¹) (Table 3). The interactions of year and genotypes, year and micronutrients, affecting Fe are presented in Figs. 1 and 2, respectively. The interaction of genotype and micronutrients affecting different measured parameters is presented in Table S1.

Correlation coefficients

The correlation coefficients indicated different traits were highly and significantly correlated. Accordingly, plant height was significantly and positively correlated with W (0.6827) and Y (0.6773). There was also a high and positive correlation between W and Y (0.9527). Pr was highly and positively correlated (0.4933) with LA. The correlations of plant growth and yield related components were significant. Crude protein was significantly and highly correlated with different measured traits except for NG. The correlation coefficients (Table 4) indicated Fe and Mn as the most and least effective micro nutrients on the growth of maize hybrid genotypes. Accordingly, Fe and Mn had correlations of 0.3137 and 0.1744 with W and 0.2456 and 0.1346 with Y. However, crop yield was significantly affected by all micro nutrients.

Table 4 Correlation coefficients among different measured traits Pearson Correlation Coefficients, $N = 448$ Prob $>|r|$ under $H_0: \text{Rho} = 0$

H	Hc	LA	W	Y	NR	NG	Pr	Fe	Zn	Mn	
H	1.00000	0.62599**	0.11789*	0.68275**	0.67739**	- 0.22336**	0.33869**	0.18439**	0.35026**	0.13577**	- 0.00079
Hc	0.62599**	1.00000	- 0.01361	0.59029**	0.57861**	- 0.13779**	0.18488**	0.20853**	0.24324**	0.08784	0.08340
LA	0.11789*	- 0.01361	1.00000	0.17808**	0.15313**	0.09526*	0.07605	0.49336**	0.23214**	- 0.05709	- 0.00787
W	0.68275**	0.59029**	0.17808**	1.00000	0.95271**	- 0.05149	0.36963**	0.35113**	0.31377**	0.24034**	0.17442**
Y	0.67739**	0.57861**	0.15313**	0.95271**	1.00000	0.06810	0.31359**	0.35472**	0.24564**	0.19633**	0.13460**
NR	- 0.22336**	- 0.13779**	0.09526*	- 0.05149	0.06810	1.00000	0.30470**	0.11733*	0.13786**	0.22827**	0.22086**
NG	0.33869**	0.18488**	0.07605	0.36963**	0.31359**	0.30470**	1.00000	- 0.02644	0.26525**	0.26349**	0.10244*
Pr	0.18439**	0.20853**	0.49336**	0.35113**	0.35472**	0.11733*	- 0.02644	1.00000	0.20415**	0.10451*	0.16059**
Fe	0.35026**	0.24324**	0.23214**	0.31377**	0.24564**	0.13786**	0.26525**	0.20415**	1.00000	0.27415**	0.26730**
Zn	0.13577**	0.08784	- 0.05709	0.24034**	0.19633**	0.22827**	0.26349**	0.10451*	0.27415**	1.00000	0.13901**
Mn	- 0.00079	0.0834	- 0.00787	0.17442**	0.13460**	0.22086**	0.10244*	0.16059**	0.26730**	0.13901**	1.00000

H (cm): maize height, Hc (cm): cob height, LA: leaf area index, W: weight of 1000 grains (g), GY (ton): grain yield, NR: number of grain rows., NG: number of grains in a row, Pr: crude protein, Zn (mg/kg): zinc, Mn (mg/kg): manganese, Fe (mg/kg): iron

Discussion

The results indicated it is possible to increase maize growth, yield, and nutrient uptake in different genotypes using the tested micronutrients. Micronutrients significantly increased maize height and cob height from the soil surface with significant differences among different genotypes. Such results indicate the effectiveness of both micronutrients and maize genotype on maize growth under micronutrient deficiency in the arid and semi-arid areas. The significant differences among the two years indicate the effects of the environment and climate on the maize genotypes. The significant interactions among micronutrients and maize genotypes and year are the indicator of different responses of maize genotypes to the use of micronutrients in the two years.

Analysis of variance

According to the analysis of variance, there were significant differences between the effects of the two years on the measured parameters, except the weight of 1000 grains and Fe uptake. This indicates there had been variation from the first year to the second year. The observed differences may be due to climatic and environmental parameters affecting the experiments in each year. Accordingly, the averaged values for the 2 years can more precisely illustrate the real effects of time affecting the measured parameters. However, interestingly, two of the most important parameters, including the weight of 1000 grains and Fe uptake, were not affected by the effects of the years. Such results indicate each year's measurement of grain yield and Fe uptake can be a suitable indicator of potential yield and Fe uptake in the region and the similar regions in the world.

Maize growth, yield, and grain protein

Micronutrients were able to increase LA in different genotypes compared with control. Due to their important functioning in plants, a suitable concentration of micronutrients including Zn, Mn and Fe are essential for the higher growth of maize genotypes under micronutrient deficiency. However, the correlation coefficients (Table 4) indicated just Fe as the most effective micro nutrient on LA.

Our results indicated that the combined effects of micronutrients were more effective on the growth, yield and nutrient uptake of maize in the two-year experiment. Such results illustrate the tested micronutrients are not antagonistic to each other and can synergistically enhance the growth, yield and nutrient uptake of maize hybrid genotypes. It is also of significance, because such a method

can contribute to the enhanced quality of maize grains (fortification) for human nutrition. Nutrient deficiency is a worldwide issue, especially in the developing continents. Accordingly, breeding the maize genotypes for higher absorption of micronutrients, resulting in higher grain yield and nutrient uptake, may be one of the most important research topics, which eventually result in the improved feeding of the world's increasing population.

Simialry, Rasool et al. (2019) examined the growth and yield of hybrid maize affected by seed priming with the single and combined use of different micronutrients (0.01, 0.1 and 0.5%) including Zn, B, and Mn, The authors found that seed priming increased maize seed germination, crop grain and biological yield, 1000-grain weight, grain rows per cob, grains per cob, cob length and grain protein content. They also indicated that the combined use of the treatments (B + Zn + Mn at 0.01 + 0.5 + 0.1%) followed by B + Zn were the most effective treatments significantly increasing maize growth, yield and quality.

Zhang et al. (2017b) indicated if maize components of oil, protein and starch are genetically regulated by breeding tools, the quality of maize grain can be improved. Accordingly, they detected the quantitative trait loci (QTL), which can control maize grain oil, protein, starch and lysine. The maize genotypes, which were examined in the experiment, consisted of 498 recombinants of inbred lines grown in six different environments. The authors found maize grain of oil, protein, starch, and lysine were controlled by a total of 13, 25, 31 and 15 QTLs, in different environments.

The significant interactions of G x M indicate that the effects of micronutrient fertilization, are different on each maize hybrid genotype. The reason can be due to the differences in the genetic combination of each hybrid genotype significantly affecting the absorption and subsequent incorporation of micronutrients in the physiological processes of each genotype. A more productive genotype has higher growth, yield and grain quality. The results indicated it is possible to improve the growth, yield and quality of the tested genotypes using the micronutrient treatments, although the responses to the treatments were significantly different in the two years. The most efficient genotype can be used for breeding purposes and subsequent enhancement of plant growth, crop yield and grain quality. For example, due to micronutrient deficiency, worldwide, fortification of cereal grains including maize can be a useful approach to provide people with their required micronutrients.

Micronutrients are able to increase grain yield (weight of 1000 grains, number of grain rows, and number of grains) by enhancing the process of photosynthesis and the translocation of photosynthates to the grains during the physiological maturity (Jadhav et al. 2020). The three types of micronutrient fertilizers may increase the yield of maize

genotypes by increasing: (1) auxin biosynthesis, (2) chlorophyll concentration, (3) the activity of phosphoenolpyruvate carboxylase and carboxylase biphosphate ribulose, and (4) N and P uptake, and (5) reducing sodium accumulation in plant tissues (Aziz et al. 2019; Liu et al. 2020).

Nutrient uptake

There were significant differences among different genotypes in the uptake of different micro-nutrients. The genetic combination of maize hybrid genotypes determines how the plant may absorb micronutrients and incorporate them into the physiological pathways and plant structure. Woli et al. (2019) investigated the effects of different maize hybrids on the uptake of micronutrients. They reported a newer hybrids contained higher nutrient concentration, especially in their reproductive parts. They attributed such a difference to the higher uptake of nutrients and higher dry matter. There were not any differences in the remobilization of nutrients from the vegetative to the reproductive tissues except for Fe and Ca. Similar to our research, Woli et al. (2019) found that there were not any significant differences among different plant tissues in terms of Mn concentration indicating that plant Mn concentration, compared with the other micronutrients, is less affected by genetic and environment.

The highest Mn concentrations were related to genotype 1 without any treatment. According to the present study, different plant tissues in terms of Mn concentration were less affected by genetics and environment, compared with the other micronutrients, however M8 plants are taller and better in grain yield than M6 (not treated with Mn). Although the single use of Mn may be less affected by genetics and environment, however, its combined use with Fe and Zn has increased plant growth and yield, which can be due to positive synergistic effects between the tested micronutrients (El-Yazal, 2019).

Micronutrients are the pre-requisite for the activity of the enzymes, which eventually result in the production of amino acids and proteins. Interestingly, the highest effects of micronutrients on grain crude protein resulted from the single effects of Mn and its combined use with Zn and Fe (Rassol et al. 2019). Halimiyan et al. (2020) also found that the foliar use of micronutrients improved the protein quality of maize grains in control and drought stress conditions.

Although the tested corn hybrids in the present research have been selected and developed for drought tolerance, another important factor, which determines the selection of such hybrids in the arid and semi-arid areas of the world, is their micro-nutrient utilization efficiency. Such a complex parameter, which is affected by root and leaf uptake of

micronutrients, and their translocation and assimilation, is a function of environmental and genetic factors. The translocation of micronutrients in the plant is controlled by transporter proteins. Accordingly, besides such proteins, the molecular genetics affecting the uptake and translocation and utilization of metals in crop plants is also of significance for breeding purposes and developing more efficient con genotypes (Moreira et al., 2018).

According to the correlation of analysis, plant height was highly and significantly correlated with maize weight of 1000 grains and yield, which indicated the optimum height of maize may result in the highest yield. Leaf area was also significantly and positively correlated with grain protein content, which is due to the important role of plant leaves in the process of photosynthesis and the production of photosynthates. The other interesting result of the presented research, which has been indicated by the correlation analyses, is the higher correlation of Fe with maize growth compared with Zn and Mn. Although the deficiency of micronutrients is usual in the calcareous soils of arid and semi-arid areas, the results indicated that maize plants are more sensitive to Fe deficiency. The production of Fe carbonates in the calcareous soils is one of the most important reasons significantly decreasing Fe availability to the plant (Elanchezhian et al. 2017).

Conclusion

Maize yield and quality were enhanced by the combined treatments of Zn, Mn and Fe at the V8 growth stage and the full appearance of the plant organs. Plant Fe concentration was not affected by the effects of the years, and micronutrients increased Fe and Zn concentration. Plant Mn concentration was not affected by the tested treatments, and it was the only micronutrient, which increased grain crude protein. Genotype 3 may be selected as the most yielding genotype in the arid and semi-arid areas. According to the results, the concentration of 3 g. L⁻¹ in the single treatments and 1.5 g. L⁻¹ in the combined treatments significantly affected maize yield and quality. There were not any antagonistic effects among the different micronutrients in the combined treatments. The significance of the presented research is due to the following: (1) increasing maize growth, yield, and nutritive value in the semi-arid areas of the world, can provide people with higher quality food, (2) the enhanced quality of maize grains due to higher rate of micronutrients and crude protein is of health and economic importance. Accordingly, one of the most important research topics is breeding maize genotypes for higher absorption of micronutrients, which results in maize plants with higher grain yield and quality.

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Declarations

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

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