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Agronomic bio-fortification of iron, zinc and selenium enhance growth, quality and uptake of different sorghum accessions

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Abstract: Agronomic bio-fortification is one of the main approaches for mitigation of micronutrient shortage in human populations and endorses sustainable production of food and feed. Studies related to agronomic bio-fortification of crops are mainly focused on single or rarely two micronutrients application, and no attempt has made to study the combined effect of zinc (Zn), iron (Fe) and selenium (Se) on forage sorghum. Therefore, this research was accomplished to evaluate the effect of Zn, Fe and Se bio-fortification on diverse sorghum accessions. The field experiments were conducted in a randomised complete block design with a split-plot arrangement. The treatments comprised of Zn (10 mg/L as $ZnSO_4·5H_2O$), Fe (7 mg/L as $FeSO_4·7H_2O$), Se (3 mg/L as $SeSO_4$) and CK (control) were applied to five sorghum accessions: G_1 (Y-16), G_2 (YSH-166), G_3 (YSH-134), G_4 (YSS-98) and G_5 (YSH-132). According to our results, the sorghum accession G_5 showed superiority over all other accessions and produced maximum values of all growth and quality traits except grains number per panicle and 1 000-grain weight. All applied micronutrients (Zn, Fe and Se) enhanced the growth, quality and uptake of nutrients in sorghum accessions. However, Se recorded the highest plant height, stem diameter, 1 000-grain weight and Zn produced the maximum protein, oil and starch contents. Conclusively, it can be concluded that G_5 with Se must be used to achieve the optimum values of agronomic traits, while G_5 with Zn found more effective to improve the quality traits of sorghum.

Keywords: *Sorghum bicolor* L. Moench; deficiency; malnutrition; bioavailability; phytate

Micronutrients such as zinc (Zn), iron (Fe), and selenium (Se) play an important role in human growth, development, and maintenance of the immune system (Shenkin 2006). Micronutrient malnutrition has always been considered a difficult task for humans (Copenhagen Consensus 2008) because 2/3rd of the world's population may deficient one or more important mineral elements (White and Broadley 2009, Stein 2010). Zinc is a crucial micronutrient that is involved in numerous biochemical paths (Alloway 2009). All around the world, about 2.7 billion people suffer from Zn deficiency (WHO 2012). On the other

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hand, Fe is one of the most essential micronutrients, and nearly two billion people suffer from iron deficiency worldwide, which has frequently been claimed to be the major cause of anemia (Welch and Graham 1999). While selenium is an indispensable trace element to humans and animals. Consequently, augmenting micronutrient concentrations in staple cereal grains through agricultural practices is measured as an effective tactic to overcome micronutrient malnutrition in humans (Hossain et al. 2021).

Micronutrient malnutrition in humans in developing countries is derived from deficiencies of these elements in staple food, such as rice or wheat in Asia and maize or sorghum in Africa (Fang et al. 2008). Sorghum has its importance due to its grains, stalks and leaves, and it is used for fodder and feed for animals other than human beings (Rooney et al. 2007). Farmers prefer its cultivation due to its drought and heat resistance in addition to soil salinity tolerance in tropical areas. Furthermore, it requires little irrigation requirements as compared to maize, and this quality makes it a favourable crop for rain-fed as well as irrigated areas (Soleymani and Shahrajabian 2012).

It is thought that increasing the micronutrient concentrations in grain crops could intensify the nutritional intake of these elements in these areas notably (White and Broadley 2005). Thus, it is very vital to increase the volume of bio-available micronutrients, which is reliant on both intake and absorption of micronutrients. Recently, many calculations of micronutrients bioavailability have been projected, such as phytate/Zn or phytate/Fe molar ratios of cereals and legumes (Chan et al. 2007). Furthermore, in spite of the advantage of phytate for human health, many methods were introduced to diminish it for improving mineral bioavailability of cereals and legumes, containing molecular genetic modification, soaking, fermentation, polishing and phytase treatment (Badau et al. 2005, Lestienne et al. 2005, Ren et al. 2007). For many reasons, however, none of these have been universally successful in solving micronutrient deficiencies in developing countries. Therefore, in our judgment, the best results of agronomic interventions should be used to aim for augmented micronutrients mass fraction, and further food processing approaches should aim at decreasing phytate while even maintaining or increasing the density of minerals in crop grain.

Among strategies to upsurge the iron, zinc and selenium levels in cereal grains, bio-fortification seems to be the most viable and cost-effective method (Cakmak 2008). Bio-fortification of primary cereals with micronutrients by using agricultural tactics, such as plant breeding and agronomic bio-fortification, characterises a useful, cost-effective and sustainable approach to overcome the micronutrient deficiencies in human populations (Zou et al. 2012, Bouis and Saltzman 2017). It provides a sustainable solution to the problem of nutritional deficiencies by exploring natural genetic variation to produce minerals-rich crop plants (Mayer et al. 2008).

The previously published research related to agronomic bio-fortification of agricultural crops is mainly focused on single or rarely two micronutrients application. To the best of our knowledge, no attempt has been made to study the combined effect of Zn, Fe and Se on forage sorghum. Therefore, the present study was performed to evaluate the impact of Zn, Fe and Se bio-fortification on their concentrations, growth, protein, oil and starch contents of various sorghum accessions to acquire a reasonable concentration of mixed micronutrients fertiliser to enrich micronutrients in sorghum grain.

MATERIAL AND METHODS

Plant material and research site. Field experiments were conducted during summer seasons, 2016 and 2017, at the experimental area of plant breeding and genetics, College of Agriculture, University of Sargodha, Sargodha, located at 32°08'N and 72°67'E, Punjab, Pakistan. Wheat was sown in this research area prior to the start of the present study. From fifteen sorghum accessions, seeds of five accessions (Y-16, YSH-166, YSH-134, YSS-98 and YSH-132) were selected based on their performance in the previous field experiment. These accessions were collected from Maize and Millet Research Institute Yousaf wala, Sahiwal, Punjab, Pakistan.

Soil characteristics and weather description. Soil samples from various depths (0–15, 15–30 and 30–45 cm) were analysed at planting. From each replication, several subsamples were taken and then bulked to one sample. Soil organic carbon was determined by using the method of Walkey-Black (Jackson 1962). The pH meter was used for the determination of soil pH. Nitrogen was determined by Ginning and Hibbard's method of H_2SO_4 digestion, and distillation was made with macro Kjeldhal's apparatus (Jackson 1962). The available phosphorus in the soil was determined by the Olsen method (Sims 2000). For potassium extraction, a 0.5 mol/L ammonium

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Table 1. Analysis of experimental soil (presented data is the average of two years)

Characteristic	Soil sample depth (cm)			
	0–15	15–30	30–45	mean
Soil pH	7.21	7.37	7.86	7.48
Organic carbon (%)	1.28	1.15	1.01	1.14
Total nitrogen (%)	0.071	0.063	0.059	0.064
Olsen's phosphorus (mg/kg)	5.37	7.35	10.21	7.64
Extractable potassium (mg/kg)	173	160	115	149
DTPA extractable Zn (mg/kg)	0.74	0.68	0.59	0.67

DTPA – diethylenetriaminepentaacetic acid

acetate/acetic acid solution was added to the air-dried soil sample and stirred with a mechanical stirrer for 30 min. This method effectively displaced potentially available K^+ ions. Then, the potassium content of the filtered extract was determined using a flame photometer. The analysis of experimental soil showed in Table 1. From 0 cm to 45 cm soil depths, soil pH and organic carbon ranged between 7.21 to 7.86 and 1.28 to 1.01%, respectively. However, the level of N was low, and P and K was medium of experimental soil (Table 1). The daily maximum or minimum temperature and rainfall data of the entire experimental phase were also noted (Figure 1). The total mean rainfall was 175 mm in 2016 and 408.1 mm in 2017 during the entire growing period of sorghum.

Experimental details and treatments. The experiments were laid out in randomised complete block design (RCBD) with a split-plot arrangement

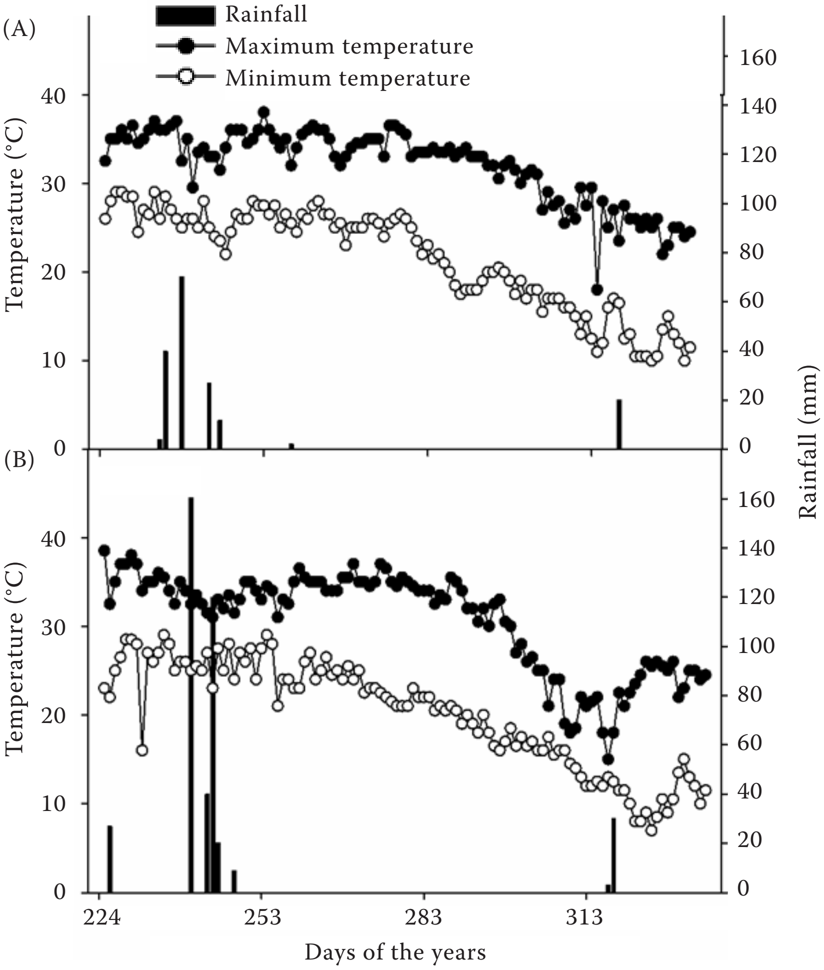

Figure 1. The daily maximum or minimum temperatures and rainfall during the sorghum growing seasons of the experimental site (A) 2016 and (B) 2017 at Sargodha, Punjab, Pakistan

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having three replications. Five sorghum accessions: G_1 – Y-16; G_2 – YSH-166; G_3 – YSH-134; G_4 – YSS-98 and G_5 – YSH-132 and four treatments: Zn – 10 mg/L as $ZnSO_4·5H_2O$; Fe – 7 mg/L as $FeSO_4·7H_2O$; Se – 3 mg/L as $SeSO_4$ and CK (control-untreated) were used in the study. In this study, the total doses of Zn (2.47 kg/ha), Fe (1.72 kg/ha) and Se (0.74 kg/ha) were supplied through two methods viz basal application and foliar spray. The net plot size was 12 m × 3 m by keeping plant to plant and row to row distances 25 cm and 75 cm, respectively. Sorghum accessions were allocated to main plots while trace elements were applied in the sub-plots. The total population of the sorghum plant was 90 000/ha. The crop was sown on 11th and 13th August 2016 and 2017, respectively. The crop was planted on well-prepared ridges, which were prepared with 2–3 cultivation using tractor followed by planking. The final ridges were prepared using a ridger. Basal dose of nitrogen, phosphorus and potassium was applied at 50 + 60 + 90 kg/ha, respectively, to all experimental plots. Hoeing was done at different intervals to keep the crop free from weeds, and three irrigations were applied from sowing to harvesting. Treatments were applied at three different growth and developmental stages: (*i*) after sowing; (*ii*) at anthesis and (*iii*) after anthesis.

Measurement and sampling

Measurement of plant traits. At the flowering stage, five sorghum plants from the middle rows of every treatment were destructively sampled. The numbers of leaves per plant were counted, and plant height was measured from base to tip with the help of a meter rod. Stem diameter was measured with the help of a vernier caliper, and then the average was taken for diameter. Leaf area index was measured in cm^2 with the help of a leaf area meter. At maturity, the number of grains per panicle and 1 000-grain weight was measured with standard procedure.

Protein, oil and starch contents (%) in grains. For quality analysis, seed samples were scanned on a near-infrared reflectance spectroscopy system (NIRS) to determine protein and oil contents. To measure starch, we followed a previously published method with minor modifications (Beta et al. 2001). Sorghum grain (100 g) was steeped in 200 mL of NaOH (0.25%, *w*/*v*) at 5 °C for 24 h. The steeped grains were washed and ground with an equal volume of water using a blender for 3 min. The slurry was filtered through a 200-mesh screen. The material remaining on the sieve was rinsed with water. Grinding and filtering were repeated on this material. After rinsing, the material still remaining on the sieve was discarded. The filtrate was allowed to stand for 1 h. The filtrate was centrifuged at 760 g for 10 min. The grey-colored, top protein-rich layer was removed using a spatula. Excess water was added to re-suspend the sample, and centrifugation was done for 5 min. Washing and centrifugation were repeated several times until the top starch layer appeared white. The starch was dried for 24 h at 40 °C.

Zn, Fe and Se uptake. At harvest, the concentration of Zn, Fe and Se was estimated from grain by following previously published methods (Wright and Stuczynski 1996). After washing with deionised water, samples were oven-dried for 48 h at 70 °C and ground into fine powder. The mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added to the samples. The concentration of Zn, Fe and Se was determined with the help of atomic absorption spectrophotometer (Melbourne, Australia).

Statistical analysis. Data of both years were non-significant and was pooled over the year. Data were analysed using the Statistix software (version 8.1., Statistix, Tallahassee, USA), and analysis of variance (ANOVA) technique was exercised to testify the overall significance of data (Steel et al. 1997). To compare the means, the least significance difference (*LSD*) test was employed at the 0.05 probability level. In addition, figures were drawn on Microsoft Excel 2013 (Washington, USA) by using standard error (± SE).

RESULTS AND DISCUSSION

Effect of Zn, Fe and Se on morphological traits of sorghum accessions. Different micronutrients and sorghum accessions significantly ($P < 0.05$) affected the growth (plant height, stem diameter, number of leaves per plant, leaf area) and yield (number of grains per plant, and 1 000-grain weight) parameters of sorghum. At maturity, the maximum plant height (135 cm) and stem diameter (11.42 cm) were determined for G_5, while the minimum plant height (121 cm) and stem diameter (7.57 cm) were recorded for G_1. Among all the treatments, the highest plant height (132 cm) and stem diameter (10.44 cm) were obtained with Se application. The interactive effects of micronutrients and sorghum accessions for plant height, stem diameter were found significant ($P < 0.05$) in both study years (Table 2). This research

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indicated that foliar and basal application of zinc (Zn), iron (Fe) and selenium (Se) impart constructed impact on the above traits.

The increase in these traits of particular accessions might be due to its genetic makeup where the basal and foliar application of Zn, Fe and Se imparts significant effects on plant height and stem diameter of sorghum. The response of different sorghum genotypes to Zn application also varied in previous studies (Markole et al. 2020). However, among the treatments, Se recorded the maximum plant height, and stem diameter may be due to timely availability and efficient utilisation of Se and other nutrients (Witold et al. 2008) that improved the catalytic action in plant and also resulted in early plant maturity. In addition, foliar application of Se alleviates the micronutrients deficiency in sorghum plants and favours biomass production (de Farias Guedes et al. 2020, Teixeira et al. 2020). Plant height and stem diameter of other plants was also enhanced by the application of trace elements in sandy soil with low fertility (Zeidan et al. 2010, Khalid et al. 2019).

The maximum number of leaves (10.25 per plant), leaf area (596.7 cm^2), number of grains (1 495 per plant), and 1 000-grain weight (20.91 g) was noticed for G_3, while the minimum number of leaves (8.66 per plant), leaf area (551.4 cm^2), number of grains (1 445 per plant), and 1 000-grain weight (18.30 g) were obtained for G_4. However, as compared to all other treatments, the highest number of grains (1 521 per plant) was obtained with Zn application, while the number of leaves (10.60 per plant), leaf area (613.13 cm^2) and 1 000-grain weight (20.51 g) were observed maximum under Se application. The interaction of micronutrients and sorghum accessions for the number of leaves per plant, leaf area, number of grains per plant, and 1 000-grain weight were found significant ($P < 0.05$) in both study years (Table 2).

The increase in these traits of sorghum might be due to more efficient use of trace elements as well as unceasing supply of Zn, Fe and Se at the time of its perilous stages of crop growth. The increased dry matter of shoots and roots has been reported in sorghum plants sprayed with Si (Flores et al. 2018). Similarly, deficiency of Zn reduced the photosynthates that ultimately affect the leaf area and crop growth rate of sorghum plants (Cakmak et al. 1989, Dambiwal et al. 2017). In addition, this increase may be attributed to the absorption of necessary nutrients (N, P and K) that enhanced crop yield and its component (Thiruppathi et al. 2001). Moreover, improved yield-related traits might be due to the production of more chlorophyll content, elevated photosynthetic rate and cell division, more translocation of assimilates towards reproductive parts than vegetative parts because of higher partitioning of assimilates towards the sink. Likewise, the increased photosynthetic pigments and protein content in the aerial parts favoured

Table 2. Effect of zinc, iron and selenium treatments on morphological traits of five sorghum accessions

		PH (cm)	SD (mm)	NL	LA	NG	GW (g)
Accession (G)	G_1	121.71^d	7.57^d	8.66^c	551.42^c	1 459.9^d	18.59^d
	G_2	123.97^c	10.07^c	9.16^b	567.75^b	1 476.9^c	18.89^c
	G_3	127.21^b	10.59^b	10.25^a	596.75^a	1 495.8^a	20.91^a
	G_4	125.43^c	10.21^c	8.75^c	559.58^{bc}	1 445.9^e	18.30^e
	G_5	135.35^a	11.42^a	9.91^a	563.50^b	1 485.4^b	20.13^b
$LSD_{0.05}$		1.71	0.21	0.39	8.97	2.00	0.07
Treatment (T)	Zn	125.52^b	9.87^b	10.13^a	593.00^b	1 521.5^a	19.14^c
	Fe	126.09^b	9.98^b	9.20^b	564.87^c	1 484.2^b	19.47^b
	Se	132.23^a	10.44^a	10.60^a	613.13^a	1 460.2^c	20.51^a
	CK	123.09^c	9.60^c	7.46^c	500.20^d	1 425.3^d	18.34^d
$LSD_{0.05}$		1.45	0.15	0.76	19.14	10.46	0.25
Interaction (G × T)		*	*	*	*	*	*

PH – plant height; SD – stem diameter; NL – number of leaves per plant; LA – leaf area; NG – number of grains per panicle; GW – 1 000-grain weight; G_1 – Y-16; G_2 – YSH-166; G_3 – YSH-134; G_4 – YSS-98; G_5 – YSH-132; Zn – 10 mg/L as $ZnSO_4 \cdot 5H_2O$; Fe – 7 mg/L as $FeSO_4 \cdot 7H_2O$; Se – 3 mg/L as $SeSO_4$; CK – control (untreated). Means with the same small letters are not significantly different at $^*P < 0.05$ in the same column. Values are means of three replicates for each treatment. *LSD* – least significant difference a more efficient use of trace elements during the initial growth phase of sorghum plants (de Farias Guedes et al. 2020). Our results are consistent with the finding of Bameri et al. (2012), who reported the high number of grains per spike, 1 000-grain weight and grain yield of wheat with the foliar application of zinc, iron and manganese. Similarly, foliar application of iron enhanced hundred-grain weight and other yield-related parameters of soybean and sorghum (Heidarian et al. 2011, Choudhary et al. 2015).

Effect of Zn, Fe and Se treatments on protein, oil and starch contents (%). In this field study, all sorghum accessions exhibited significant ($P < 0.05$) differences in seed protein content. In both years, the mean highest (9.58%) and lowest (6.36%) protein content in sorghum seed was noted with accessions G_5 and G_1, respectively. Additionally, the application of different micronutrients significantly ($P < 0.05$) impacted the seed protein content in sorghum seeds. The maximum seed protein content (9.63%) was measured under Zn treatment, and the minimum seed protein content (8.07%) was determined under the control treatment. The interactive effect of sorghum accessions and micronutrients treatments for protein accumulation was found significant ($P < 0.05$) in both years (Figure 2A). Compared with control, the oil content in sorghum seeds was enhanced with different micronutrients (i.e., Zn, Fe, and Se) application. The highest (12.03%) oil content in sorghum seeds was observed under Zn, and the lowest (10.31%) oil content in sorghum seeds was measured in the control treatment. While, among different sorghum accessions, the maximum (12.17%) and minimum (9.08%) oil content in sorghum seeds was noticed with accession G_5 and G_1, respectively. Moreover, the interactive effects between sorghum accessions and micronutrients treatments were found significant ($P < 0.05$) in both years (Figure 2B). The percentage of protein and oil in sorghum seeds are the fundamental components in defining the quality of sorghum meal and their market value (Kulamarva et al. 2009). The optimum values of protein and oil contents with Zn might be due to the effects of Zn on protein synthesis and protein functions (Broadley et al. 2007). In earlier studies, the foliar application of Zn had a positive effect on the yield and quality of forage sorghum by increasing ash and protein percentage (Soleymani and Shahrajabian 2012). In the present study, the application of Zn, Fe and Se increased the protein and oil content in sorghum seeds. Similarly, a positive correlation between grain protein and Zn concentration was also reported in wheat, soybean and mung bean (Cakmak et al. 2010, Ali et al. 2014, Malakooti et al. 2017).

The highest (52.76%) starch content was estimated in accession G_5, while the lowest (44.70%) starch content was noticed with G_1. Application of different micronutrient resulted in the increment of starch accumulation in sorghum seeds. Specifically, in this study, the high-

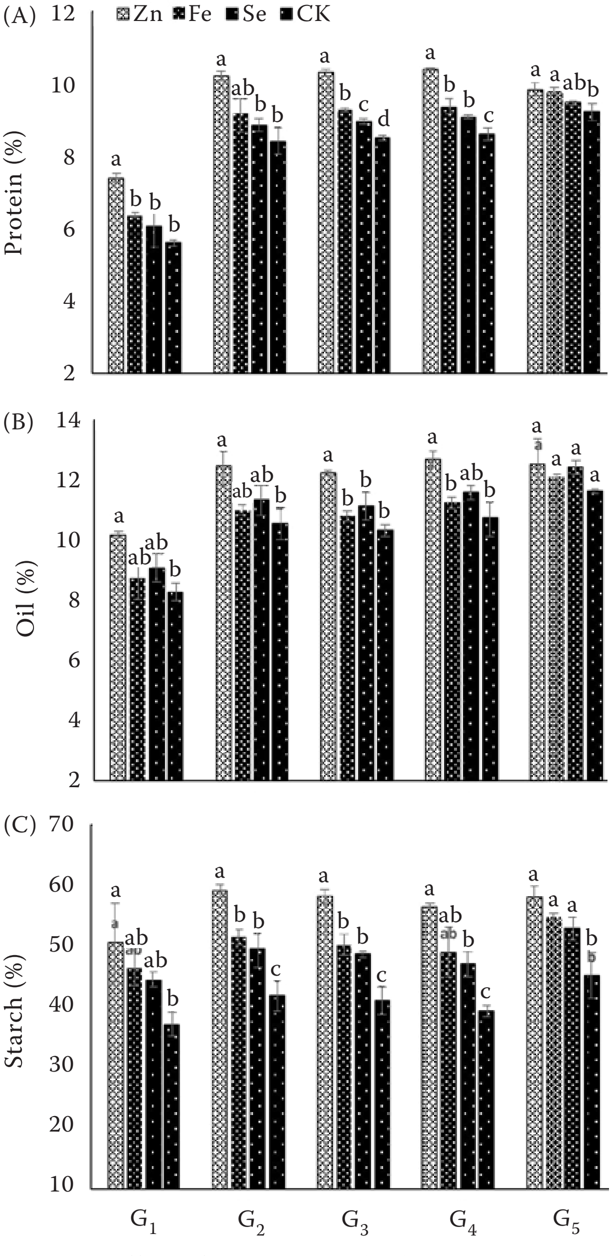

Figure 2. Effect of zinc, iron and selenium treatments on (A) protein; (B) oil and (C) starch of five sorghum accessions (presented data is the average of two years). G_1 – Y-16; G_2 – YSH-166; G_3 – YSH-134; G_4 – YSS-98; G_5 – YSH-132; Zn – 10 mg/L as $ZnSO_4·5H_2O$; Fe – 7 mg/L as $FeSO_4·7H_2O$; Se – 3 mg/L as $SeSO_4$; CK – control (untreated)

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est (56.70%) and lowest (40.91%) starch content was measured under Zn and control treatment, respectively. Furthermore, the interactive effect of sorghum accessions and micronutrients treatments for starch accumulation was found significant ($P < 0.05$) in both years (Figure 2C). In this research, all sorghum accessions exhibited significant differences in starch with the Zn. The optimum values of starch contents with Zn might be due to that most of the Zn is transported symplastically across the root to the xylem, although a substantial fraction may traverse the root and reach the xylem *via* the apoplast (White et al. 2002, Broadley et al. 2007).

Effect of Zn, Fe and Se treatments on Zn, Fe and Se uptake. Different micronutrients and sorghum accessions significantly ($P < 0.05$) affected the uptake of Zn, Fe and Se in sorghum. At maturity, the maximum uptake of zinc (25.97 g/ha), iron (39.73 g/ha) and selenium (6.53 g/ha) was observed for G_5 with Zn, Fe and Se, respectively. In contrast, the minimum uptake of zinc (13.96), iron (25.03) and selenium (1.96) was obtained for G_1 under control treatment. Furthermore, the interactive effects of micronutrients and sorghum accessions for zinc, iron and selenium uptake were found significant ($P < 0.05$) in both study years (Figure 3). The maximum Zn, Fe and Se uptake observed in this study is attributed to both adequate supply of trace elements at experimental plot and higher dry matter production. The deficiency of these elements induces physiological responses such as the degradation of cell membranes due to increased reactive oxygen species (Alloway 2008). Therefore, under micronutrient deficiency conditions, the application of micronutrients can increase the availability of these micronutrients and improve the growth of sorghum plants. Foliar application of Se increased micronutrients accumulation in sorghum plants due to its vital role in transporting trace elements to the vacuole in plant cells (Marschner et al. 1990). Similarly, increased concentration of micronutrients was observed in mung bean, soybean and sorghum with the application of trace elements at vegetative and reproductive stages (Ali et al. 2014, Malakooti et al. 2017, Oliveira et al. 2020). The foliar application of trace elements promotes the micronutrients contents in plant vegetative tissues that are available for re-translocation into seeds (Phattarakul et al. 2012). It is a fact that trace elements are more simply transported *via* phloem when applied to leaves, as occurs in the xylem when applied through roots (Poggi et al. 2000). It indicates that foliar application of trace elements plays an important role in long-distance transport within phloems, such as increased protein synthesis due to increased RNA content and cell division associated with increased indoleacetic acid in sorghum (Marschner 1995, Soleymani and Shahrajabian 2012). Therefore, stimulated transport of micronutrients into seeds might be related to the significant increase in protein biosynthesis during the early stage of seed formation (Martre et al. 2003, Ozturk et al. 2006).

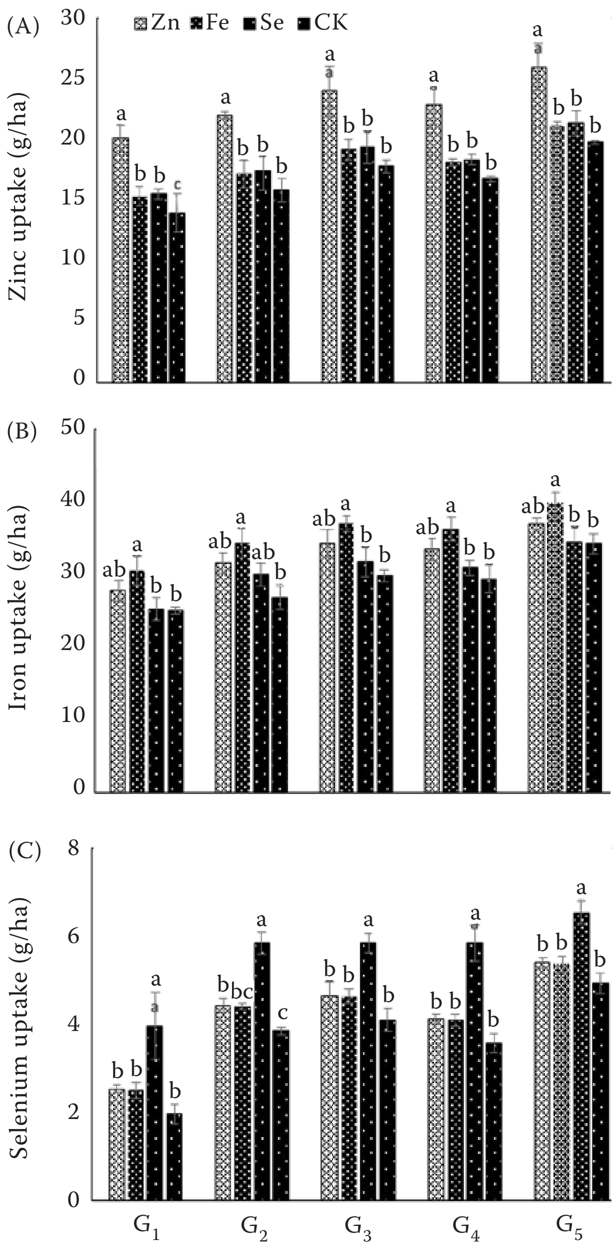

Figure 3. Effect of zinc, iron and selenium treatments on (A) Zn; (B) Fe and (C) Se uptake (presented data is the average of two years). G_1 – Y-16; G_2 – YSH-166; G_3 – YSH-134; G_4 – YSS-98; G_5 – YSH-132; Zn – 10 mg/L as $ZnSO_4 \cdot 5H_2O$; Fe – 7 mg/L as $FeSO_4 \cdot 7H_2O$; Se – 3 mg/L as $SeSO_4$; CK – control (untreated)

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It is concluded that bio-fortification of Zn, Fe and Se impart constructed impacts on all tested sorghum accessions. Among all accessions, the G_5 performed better and produced the highest values of all growth and quality traits except the number of grains per panicle and 1 000-grain weight. All micronutrients (Zn, Fe and Se) improved the growth, quality and uptake of nutrients. However, Se produced the highest plant height, stem diameter and 1000-grain weight, while Zn recorded the maximum protein, oil and starch contents in tested sorghum accessions. Therefore, Se with G_5 must be used to achieve the optimum values of agronomic traits, while Zn with G_5 should be used to enhance the quality traits of sorghum.

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