



### Article Genotypic Response of Finger Millet to Zinc and Iron Agronomic Biofortification, Location and Slope Position towards Yield

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Abstract: The present study aimed to investigate the influence of genotypic differences on responses to zinc and iron agronomic biofortification among yields of finger millet. A field experiment was conducted over two seasons in farmers' fields in Ethiopia (2019, 2020). The experimental design had 15 treatment combinations comprising three finger millet genotypes and the applications of different combinations of zinc and iron mineral fertilizers. Five soil-applied fertilizer treatments (20 kg  $h^{-1}$  $FeSO_4 + 25 \text{ kg h}^{-1} \text{ ZnSO}_4 + \text{NPKS}, 25 \text{ kg ha}^{-1} \text{ ZnSO}_4 + \text{NPKS}, 20 \text{ kg ha}^{-1} \text{ FeSO}_4 + \text{NPKS}, \text{NPKS},$ and 30% NPKS) at two locations (Gojjam and Arsi Negelle, Ethiopia) and using two slope positions (foot and hill) were replicated four times in a randomized complete block design. Grain yield and biomass were evaluated on a plot basis. Plant height, total and productive tiller number, finger length of the longest spike and number of fingers per main ear were measured at the maturity stage. The combined soil application of FeSO47H2O and ZnSO47H2O increased the yield of the Meba genotype by 51.6%. Additionally, ZnSO<sub>4</sub>7H<sub>2</sub>O fertilizer application increased the yield of the Urji genotype by 27.6%. A yield enhancement of about 18.3% of the Diga-01 genotype was achieved due to the FeSO<sub>4</sub>7H<sub>2</sub>O fertilizers' application. The findings of the present study suggest that the influence of Zn and Fe agronomic biofortification on the yield of finger millet could be affected by genotype differences and environmental conditions.

Keywords: agronomic biofortification; finger millet; genotype; iron; yield; zinc

#### 1. Introduction

Cereal crops naturally have very low grain zinc (Zn) and iron (Fe) concentrations, and growing them on soils deficient in potentially plant-available Zn- and Fe further affects yield as well as grain Zn and Fe concentrations [1]. Studies show that about half of cereal-cultivating soils globally are deficient in plant-available Zn [2], particularly acidic soils, and those in high-rainfall areas of the tropics. In spite of high total concentrations of Fe in tropical soils, high-level oxidation and fixation significantly affect their plant availability [3]. Deficiencies of Fe and Zn in soil results in reduced crop yield as well as quality since both minerals play major biological roles in plants, such as maintaining proper metabolic and physiological cellular processes [3].

Crop genotypes' breeding for resistance to Zn and Fe deficiency is a realistic and longterm solution to overcome problems related to Zn and Fe deficiency in soils [4]. However, breeding genotypes can require substantial time [5] as well as a relatively higher investment as compared to agronomic biofortification [6].



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Fertilization with Zn and Fe is a common practice to help to combat Zn and Fe deficiencies as a short-term strategy [3,7]. Crop Zn and Fe deficiencies are most frequently amended by agronomic biofortification through soil application of Zn and Fe fertilizers [8]. Zinc sulphate (ZnSO<sub>4</sub>) and ferrous sulphate (FeSO<sub>4</sub>) are used extensively as sources of Zn and Fe fertilizers, because of their higher solubility in water and existence in both crystalline and granular forms [9]. Many studies have reported that the agronomic biofortification with Fe and Zn positively enhances crop yield and/or grain Fe and Zn concentrations in crops like wheat, maize and rice [1–3].

Crop selection in agronomic biofortification plays a critical role in its effectiveness. The identification and improvement of traditional or native crops that are highly adaptive to local climates and that can efficiently withstand biotic and abiotic stresses is crucial. Finger millet (*Eleusine coracana* L.) represents one of the critical plant genetic resources for the agriculture and food security of populations inhabiting arid, infertile and marginal lands [10]. In the semiarid tropics of eastern Africa, it is the major staple food for millions of resource-poor people and plays an important nutritional and economic role [11]. Finger millet is adaptable to adverse agro-ecological conditions with minimal inputs (fertilizer, pesticides and herbicides), showing low moisture stress and disease tolerance, is productive on marginal land where other crops cannot perform, and shows tolerance to acidic soil [12,13]. In Ethiopia, finger millet is the sixth most important crop after tef, wheat, maize, sorghum and barley. It has been produced on 480,343 hectares of land, from which ~1.2 M tons have been obtained at the national level per year [14]. Nationwide, ~1.55 M households are directly engaged in finger millet production, and the production has increased by 300% in the previous 20 years [14].

Previous reports on the impact of agronomic biofortification with Zn and Fe, regarding genotype as well as slope position, of indigenous crops like finger millet and teff in tropical smallholding farming systems are lacking. However, a report from Ethiopia has indicated that wheat yield was more strongly influenced by slope positions than either the nutrient sources or rates; thus, site-specific fertilizer treatment is strongly recommended [15]. Therefore, this paper reports on the effect of basal application of Zn and Fe fertilizer on grain yield and the yield attributes of three finger millet genotypes at different locations and slope positions.

#### 2. Materials and Method

#### 2.1. Field Experiment

The agronomic biofortification trials with Zn and Fe micronutrients were carried out at the Gojjam (11°41′54′′ N 37°29′79′′ E foot slope and 11°40′23′′ N 37°30′29′′ E hill slope) and Arsi Negelle (7°19′38′′ N 38°38′54′′ E foot slope and 7°18′43′′ N 38°39′57′′ E hill slope) areas on farmers' land (Figure 1). According to the classification of the agro-ecological zonation of Ethiopia, both sites are characterized as sub-humid midlands located between 1500–2300 m.a.s.l. and as having unimodal rainfall, with an average annual rainfall of 800–1200 mm [16]. The average temperature was recorded as 13 °C and 10 °C for the minimum and 23 °C and 26 °C for the maximum for Arsi Negelle and Gojjam, respectively, during the experimental seasons. The average temperature was 17.5 °C [17] and 20 °C [18] for Arsi Negelle and Gojjam, respectively. The experiment was laid out in a randomized complete block design (RCBD) (Supplementary Materials) with a factorial concept with 4 replications consisting of 15 treatment combinations involving 3 finger millet genotypes (Dagi-01, black in colour; Urji, white in colour and Meba, brown in colour) and 5 levels of fertilizer application (Table 1).



Figure 1. Map showing the study sites locations in Amhara and Oromia regions of Ethiopia.

Treatments	Zn	Fe	Ν	Р	S	К
T1	5.5	4	32.1	3.59	15.89	31.2
T2	5.5	-	32.1	3.59	7.64	31.2
T3	-	-	32.1	3.59	5.24	31.2
T4	-	-	9.63	1.1	1.57	9.36
T5	-	4	32.1	3.59	13.49	31.2

Table 1. Elemental application of nutrients in kg per hectare.

T1: 25 kg ZnSO<sub>4</sub>7H<sub>2</sub>O, 20 kg FeSO<sub>4</sub>7H<sub>2</sub>O, 131 kg NPS, 60 kg K, 54 kg urea ha  $^{-1}$ ; T2: 25 kg ZnSO<sub>4</sub>7H<sub>2</sub>O, 131 kg NPS, 60 kg K, 54 kg urea ha  $^{-1}$ ; T3: 131 kg NPS, 60 kg K, 54 kg urea ha  $^{-1}$ ; T4: 30% of T3; T5: 20 kg FeSO<sub>4</sub>7H<sub>2</sub>O, 131 kg NPS, 60 kg K, 54 kg urea ha  $^{-1}$ .

#### 2.2. Agronomic Management

The plot size was 4 m  $\times$  4 m, with gangways between plots being 1 m wide while the distances between the blocks and the borders were 0.5 m each. The experiment was repeated for two seasons, but only at Arsi Negelle (due to COVID-19 pandemic travel restrictions), and different farms were used in each year, sowed between mid-June and mid-July and harvested in November. Planting was done by hand drilling at a seed rate of 7 kg ha<sup>-1</sup>. Each experimental plot had ten rows with 40 cm of inter-row spacing. NPKS, ZnSO<sub>4</sub>7H<sub>2</sub>O and FeSO<sub>4</sub>7H<sub>2</sub>O were applied at planting and urea was applied after 45 days at first weeding. All plots were weeded at least six times using human labour and no pesticides or herbicides were applied. Plant samples for data collection were tagged right after 100% crop germination and the crop was developed in a rainfed fashion, without irrigation.

#### 2.3. Soil Sample Collection

The soil classes of both experimental locations, including the slopes, were mostly Nitisols [17,18]. Soil samples were taken from a 60 m<sup>2</sup> circular plot in the experimental field. Five sub-sample sites were located; the first was at the centre. Two sub-sample points were selected at locations on a line through the plot centre along the crop rows, and two on a line orthogonal to the first through the plot centre. The 'long' axis of the sample array (with sample locations at 5.64 and 4.89 m) was oriented in the direction of crop rows, with the 'short axis' (with sample locations at 3.99 and 2.82 m) perpendicular to the crop rows. A single soil sub-sample was collected at each of the five sub-sample points with a Dutch auger with a flight of length 150 mm and diameter of 50 mm. Any plant material adhering to the auger was carefully removed, and the five sub-samples were stored in a single bag [19].

#### 2.4. Soil Mineral Analysis

Soil sample digestion was performed following aqua regia digestion for the extraction of trace elements method (ISO 11466) (ISO, 1995). Briefly, 3 mL of 36% HCl and 9 mL of 70%  $HNO_3$  were added in a microwave digestion tube containing 0.5 g of a ground soil sample. The digestion vessels were then transferred to the cavity of the microwave unit and the digestion was started at 185 °C and continued for 30 min. The digested sample supernatant was filtered carefully and diluted to 50 mL in a volumetric flask. CRM Wageningen-WEPAL ISE-850 (Calcareous soil) was used as a certified laboratory reference material and % mineral recovery was calculated. Blanks were also analysed at the same time. A three-step sequential extraction scheme for the fractionation of sulphur (S) was followed, using 0.01 M KNO<sub>3</sub>, 0.016 M KH<sub>2</sub>PO<sub>4</sub> and 10% tetra methyl ammonium hydroxide (TMAH) to determine soluble, exchangeable and organically-bound S fractions, respectively. The detailed procedure for soil sample collection, mineral analysis and three-step sequential extraction is reported elsewhere [19]. The soil mineral concentrations of each experimental site are presented in Table 2. The total calcium, potassium, boron, sulphur and iron contents of the soil samples were significantly different among the two locations and slope positions. The recovery for all minerals is between the acceptable ranges (85–120%).

#### 2.5. Agronomic Data Collection

The plant height, total tiller number, productive tiller number (number of basal tillers which bear mature ears), finger length of the longest spike and number of fingers per main ear were measured at the 50% maturity stage, which is after ~130 days after germination (International Board for Plant Genetic Resources [20]). Grain yield at 12% average moisture and biomass at 18% average moisture were taken (from the eight central rows) on a plot basis and then converted to a hectare basis. The plant height was measured from the ground to the tip of the inflorescence (ear). Finger length was measured from the base to the tip of the longest spike on the main tiller.

#### 2.6. Data Analysis

Data collected for all agronomic quantitative characters were subjected to analysis of variance (ANOVA) using R software version 3.3.2 (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing). The major descriptive statistics such as mean, range and standard deviation of each trait for the study genotypes, fertilizer levels, study location and slope positions were computed. Slope, fertilizer level and genotype were treated as fixed effects, whereas season, block within farm, farm within location and location were treated as random effects. Yield and biomass data were transformed to natural logarithms, as the dispersion of the random effects on the original scale appeared to increase with the fitted value. A comparison of means was done using Tukey's post hoc test (p < 0.001, 0.01, 0.05 and 0.1).

Location	Slope	В	Mg	Р	S	К	Ca	Fe	Zn
Arsi Negelle	Foot	$3.9\pm0.79~^{\rm A}$	$2052\pm47$	$2061\pm49$	$122.7\pm0.4$	$3227\pm185~^{\rm A}$	$4662\pm481~^{\rm A}$	$26918\pm1149~^{\rm A}$	$89\pm7$
	Hill	$3.0\pm0.70$ <sup>B</sup>	$1728\pm240$	$1725\pm240$	$105.8\pm7.3$	$2729\pm448^{\rm \ B}$	$4050\pm918\ ^{\rm B}$	$23952 \pm 1804$ <sup>B</sup>	$105\pm10$
	Mean	$3.4\pm0.95$ $^{\rm a}$	$1890\pm259~^{a}$	$1893\pm264~^{\rm a}$	$114.3\pm10.3$ $^{\rm a}$	$2978\pm464~^{a}$	$4356\pm870~^{\rm a}$	$25435\pm2320~^{a}$	$97\pm13$ <sup>a</sup>
	Foot	$1.0\pm0.37^{\rm \ C}$	$1731 \pm 131$	$1731 \pm 131$	$136.6\pm6^{\text{ C}}$	$934\pm33\ ^{\rm C}$	$1185\pm149^{\text{ C}}$	$107973 \pm 3372$ <sup>C</sup>	$81\pm4$
Gojjam	Hill	$0.1\pm0.08$ <sup>D</sup>	$1597 \pm 167$	$1597 \pm 167$	$207.1\pm42.8\ ^{\rm D}$	$859\pm85$ <sup>D</sup>	$1668\pm430^{\rm \ D}$	$124304 \pm 5913 \ ^{\rm D}$	$104\pm11$
	Mean	$0.55\pm0.5$ <sup>b</sup>	$1664\pm180~^{\rm a}$	$1664\pm180~^{\rm a}$	$171.8\pm47.3$ <sup>b</sup>	$897\pm82$ <sup>b</sup>	$1426\pm440~^{\rm b}$	$116138 \pm 10383 \ ^{\rm b}$	$92\pm16$ <sup>a</sup>

**Table 2.** Mineral concentrations (mg kg<sup>-1</sup>) of soil from the finger millet agronomic biofortification experimental sites in Ethiopia.

Note: Results labelled with different small letters are significantly different for location and those with different capital letters are significantly different for slope at the 0.05 probability level.

### 3. Result

Yield and Biomass Effects

Finger millet yield and biomass per hectare for each fertilizer treatment, genotype, location and slope position are presented in Figures 2–5. The total and productive tiller numbers, number of fingers per main ear, plant height and finger lengths (cm) for each fertilizer treatment, genotype, location and slope are presented in Tables 3 and 4. Yield and biomass showed a wide variation, ranging from 94 to 3828 kg and from 6.25 to 242.97 quintals per hectare, respectively. The maximum yield of the NPKS at the recommended rate in the current experiment (3594 kg ha<sup>-1</sup>) was much higher than the national average [14] of 2504 kg. The finger number per main ear, total tiller number and productive tiller number ranged between 1 and 14, 1 and 16 and 1 and 14, respectively. The plant height and finger length ranged between 4 and 120 and 3 and 17 cm, respectively.



**Figure 2.** Effect of fertilizer treatment on yield (kg) of finger millet genotypes at Arsi Negelle as affected by slope position (average data for two seasons). T1: 25 kg  $ZnSO_47H_2O$ , 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T2: 25 kg  $ZnSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T3: 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg NPS, 60 kg K, 5



**Figure 3.** Effect of fertilizer treatment on yield (kg) of finger millet genotypes at Gojjam as affected by slope position. T1: 25 kg ZnSO<sub>4</sub>7H<sub>2</sub>O, 20 kg FeSO<sub>4</sub>7H<sub>2</sub>O, 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T2: 25 kg ZnSO<sub>4</sub>7H<sub>2</sub>O, 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T3: 131 kg NPS, 60 kg K, 54 kg urea ha<sup>-1</sup>; T4: 30% of T3.



**Figure 4.** Effect of fertilizer on biomass (quintal) of finger millet genotypes at Arsi Negelle as affected by slope position (average data for two seasons). T1: 25 kg  $ZnSO_47H_2O$ , 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T2: 25 kg  $ZnSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T3: 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3; T5: 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg  $ha^{-1}$ .



**Figure 5.** Biomass (quintal) of finger millet genotypes at Gojjam as affected by zinc and iron fertilization. T1: 25 kg  $ZnSO_47H_2O$ , 20 kg  $FeSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T2: 25 kg  $ZnSO_47H_2O$ , 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T3: 131 kg NPS, 60 kg K, 54 kg urea  $ha^{-1}$ ; T4: 30% of T3.

			Foot	Slope					Hill Slope		
Genotype	Fertilizer	Total Tiller Number	Productive Tiller Number	Finger No/Main Ear	Plant Height (cm)	Finger Length (cm)	Total Tiller Number	Productive Tiller Number	Finger No/Main Ear	Plant Height (cm)	Finger Length (cm)
	T1	$4.58 \pm 1.88$	$4.4 \pm 1.9$	$6.0 \pm 1.4$	$68.6 \pm 14.2$	$8.0 \pm 1.6$	$4.8 \pm 1.7$	$4.5\pm1.7$	$5.8 \pm 1.8$	$54.3\pm8.8$	$7.3 \pm 1.3$
	T2	$4.54 \pm 1.49$	$4.1 \pm 1.3$	6.5 <u>+</u> 2	$71.8\pm21.6$	$7.6 \pm 1.5$	$5.1 \pm 1.5$	$4.7\pm1.7$	$5.8 \pm 1.4$	$56.9\pm7.6$	$7.4 \pm 1.3$
Diga-01	T3	$4.46 \pm 1.72$	$4.2\pm1.6$	$6.4 \pm 1.7$	$70.8\pm16.2$	$8.1\pm1.7$	$5.0 \pm 1.9$	$4.7\pm1.7$	$5.4 \pm 1.4$	$57.2\pm7.7$	$7.3\pm1.3$
	T4	$5.63 \pm 2.22$	$5.4\pm2.2$	$6.3\pm1.6$	$57.2 \pm 12$	$7.6 \pm 1.3$	$4.5\pm1.4$	$4.2\pm1.2$	$5.2 \pm 1.1$	$49.9\pm7.3$	$7.6\pm1.3$
	T5	$5.5\pm1.9$	$5.0\pm1.6$	$6.0\pm2.4$	$58.1\pm8.8$	$8.0\pm1.5$	$5.2\pm2$	$4.8\pm1.9$	$4.4\pm0.8$	$54.4\pm7.6$	$7.2\pm1.4$
	T1	$4.38 \pm 1.58$	$3.9\pm1.6$	$7.1\pm2.2$	$64.6\pm16.4$	$7.7\pm1.4$	$4.9\pm1.9$	$4.1 \pm 1.5$	$7.3\pm1.8$	$52.1\pm7.2$	$7.9 \pm 1$
	T2	$4.86 \pm 1.74$	$4.3\pm1.5$	$7.9 \pm 1.8$	$65.6 \pm 18.2$	$8.3\pm1.5$	$4.9\pm2.3$	$4.3\pm1.7$	$7.4 \pm 1.8$	$51.8\pm8.3$	$7.6\pm1.4$
Urji	T3	$4.81 \pm 1.96$	$4.3\pm2$	$7.6\pm1.6$	$64.8\pm13.4$	$7.9\pm1.7$	$5.4\pm2$	$4.5\pm1.4$	$7.2 \pm 1.9$	$53.0\pm8.3$	$7.4 \pm 1.3$
	T4	$5.6\pm2.9$	$5.2\pm2.8$	$7.8 \pm 1.5$	$56.2\pm13.7$	$8.3\pm1.7$	$5.6\pm2.6$	$4.9\pm2.5$	$7.0 \pm 1.6$	$47.1\pm 6.8$	$6.6\pm1.2$
	T5	$5.0\pm2.2$	$4.1\pm1.9$	$6.7\pm1.3$	$49.0\pm9.7$	$7.5\pm2$	$5.4\pm3$	$4\pm1.8$	$6.8\pm1.8$	$48.4\pm7.9$	$6.8\pm1.7$
	T1	$4.94\pm2.2$	$4.1\pm1.6$	$5.8 \pm 1.3$	$63.5\pm12.8$	$5.8\pm1.1$	$5.3\pm2$	$4.5\pm1.6$	$5.7\pm1.7$	$56.0\pm7.3$	$5.4 \pm 1.1$
	T2	$5.35\pm2.8$	$4.5\pm1.6$	$6.1\pm1.6$	$65.7\pm11$	$6.2 \pm 1.3$	$5.2\pm1.9$	$4.3\pm1.6$	$5.6 \pm 1.2$	$53.6\pm9$	$5.7\pm0.9$
Meba	T3	$5.06 \pm 1.66$	$4.5\pm1.5$	$6.2 \pm 1.1$	$64.0\pm17.1$	$5.8 \pm 1.1$	$5.2\pm1.9$	$4.1\pm1.6$	$5.6 \pm 1.2$	$53.3\pm8.6$	$5.6 \pm 1.1$
	T4	$5.0\pm2.2$	$4.7\pm2.1$	$6.0 \pm 1.4$	$56.8 \pm 12.7$	$5.7 \pm 1.1$	$5.9\pm2.4$	$4.6\pm1.9$	$5.9 \pm 1.4$	$47.0\pm8.9$	$5.2\pm0.8$
	T5	$4.7\pm1.4$	$4.0\pm1$	$5.6\pm1.3$	$50.4\pm6.9$	$5.3\pm0.8$	$7.2\pm2$	$5.8\pm2.1$	$5.9\pm1.2$	$44.8\pm12.5$	$5.4\pm0.9$

**Table 3.** Effect of fertilizer treatment on yield traits of finger millet genotypes at Arsi Negelle as affected by slope position.

**Table 4.** Effect of fertilizer treatment on yield traits of finger millet genotypes at Gojjam as affected by slope position.

Foot Slope									Hill Slope		
Genotype	Fertilizer	Total Tiller Number	Productive Tiller Number	Finger No/Main Ear	Plant Height (cm)	Finger Length (cm)	Total Tiller Number	Productive Tiller Number	Finger No/Main Ear	Plant Height (cm)	Finger Length (cm)
	T1	$1.35\pm0.6$	$1.31\pm0.6$	$6.1 \pm 1.5$	$65.1 \pm 17.1$	$9.8\pm1.6$	$1.56\pm0.7$	$1.45\pm0.7$	$5.7\pm1$	$46.5\pm6$	$9.2\pm1.1$
Digo 01	T2	$1.41\pm0.8$	$1.34\pm0.8$	$5.3 \pm 1.2$	$61.3 \pm 12.9$	$9.6\pm1.9$	$1.83\pm0.9$	$1.77\pm0.9$	$5.2 \pm 0.8$	$44.2\pm7$	$9.5\pm1.9$
Diga-01	T3	$1.38\pm0.6$	$1.31\pm0.6$	$5.8 \pm 1.2$	$66.2\pm9.1$	$9.8 \pm 1.5$	$1.45\pm0.7$	$1.39\pm0.7$	$5.2 \pm 1.2$	$44.0\pm6.9$	$8.8 \pm 1.1$
	T4	$1.36\pm0.4$	$1.29\pm0.4$	$5.1\pm0.9$	$57.4 \pm 10.8$	$9.7\pm1.9$	$1.44\pm0.6$	$1.35\pm0.6$	$4.8\pm1$	$42.3\pm10.4$	$7.7\pm1.4$
T T::	T1	$1.29\pm0.5$	$1.22\pm0.5$	$7.3\pm2.1$	$66.0\pm15.2$	$9.9\pm2.1$	$1.77\pm0.8$	$1.67\pm0.8$	$7.4 \pm 1.1$	$48.4\pm7.2$	$9.9\pm1.7$
	T2	$1.38\pm0.6$	$1.27\pm0.6$	$8.1 \pm 1.8$	$77.4 \pm 19.1$	$10.4 \pm 1.7$	$2.2\pm1.8$	$1.98 \pm 1.8$	$7.2\pm0.9$	$49.9\pm5$	$10.1\pm1.4$
OIJ	T3	$1.41\pm0.8$	$1.35\pm0.8$	$7.6\pm1.7$	$70.5\pm13.5$	$10.0\pm1.9$	$1.56\pm0.8$	$1.45\pm0.8$	$6.6 \pm 1.7$	$47.5\pm8.1$	$9.5\pm1.1$
	T4	$1.34\pm0.5$	$1.28\pm0.5$	$6.1\pm1.9$	$52.4 \pm 18.2$	$9.0\pm1.8$	$1.9\pm1.2$	$1.85\pm1.2$	$6.2\pm1.8$	$42.4\pm9.6$	$8.8\pm1.3$
	T1	$1.24\pm0.5$	$1.17\pm0.5$	$4.8\pm0.8$	$70.3\pm7.9$	$7.6\pm1.4$	$1.74\pm1$	$1.67\pm1$	$4.7\pm0.7$	$60.1\pm7.6$	$7.5\pm1.6$
241	T2	$1.33\pm0.6$	$1.25\pm0.6$	$4.9\pm1$	$69.0 \pm 11.7$	$8.2\pm1.4$	$1.55\pm0.6$	$1.45\pm0.6$	$4.7\pm1.2$	$52.0\pm9.9$	$6.7\pm2.2$
Meba	T3	$1.42\pm0.7$	$1.37\pm0.7$	$4.8\pm1$	$70.4 \pm 11.1$	$7.5\pm1.5$	$1.5\pm0.9$	$1.5\pm0.9$	$5.0 \pm 1.2$	$57.3\pm9.2$	$7.4\pm2$
	T4	$1.44\pm0.7$	$1.38\pm0.7$	$4.7\pm1.5$	$60.3\pm11$	$7.2\pm1.3$	$1.8\pm1.2$	$1.8\pm1.2$	$4.2\pm1.1$	$51.3\pm9.2$	$6.9\pm1.6$

A significant response from the Meba genotype to the combined  $FeSO_47H_2O$  and  $ZnSO_47H_2O$  fertilizer application at the Gojjam hill slope was exhibited; the average yield was increased by 51.6% (Table 5). The Diga-01 genotype responded significantly to  $FeSO_47H_2O$  fertilizers at the Arsi Negelle hill slope position; an 18.3% average yield enhancement was recorded. A significant response was observed from the Urji genotype to  $ZnSO_47H_2O$  fertilizers at the Gojjam hill slope position; a 27.6% average yield increase was observed. Irrespective of locations, slope position and genotype, grain yield was enhanced by 20% due to the soil application of  $FeSO_47H_2O$  fertilizer.

	Sum Sq	Mean Sq	NumDF	DenDF	F Value	Pr(>F)
Slope position	0.787	0.787	1	3.005	9.0260	0.05734 #
Fertilizer	7.2168	1.8042	4	274.966	20.6909	$6.382  imes 10^{-15}$ ***
Genotype	10.3013	5.1506	2	274.491	59.0686	$<2.2  imes 10^{-16}$ ***
Fertilizer:genotype	0.8588	0.1073	8	275.993	1.2311	0.2807

Significance codes: \*\*\* <0.001; # < 0.1.

Strong evidence (p < 0.001) was exhibited for the fertilizer's effect on finger millet yield irrespective of location, slope and genotype (Table 5). Similarly, the genotype shows strong evidence (p < 0.001) of effect on yield over location, slope and fertilizer. Moderate evidence (p < 0.05) has been seen for a slope position effect on finger millet yield irrespective of location, fertilizer application and genotype (Table 5). However, there is no evidence for the effect on yield due to the interaction of fertilizer and genotype (Table 5).

The fertilizer shows strong evidence (p < 0.001) of effect on finger millet biomass irrespective of location, slope and genotype (Table 6). Some evidence (p < 0.05) has been seen for a slope position effect on biomass irrespective of location, fertilizer and genotype (Table 6). Similarly, a slight genotype effect (p < 0.05) on biomass irrespective of location, slope and fertilizer was observed. However, the interaction of fertilizer and genotype exhibited no effect on biomass (Table 6).

	Sum Sq	Mean Sq	NumDF	DenDF	F Value	<b>Pr(&gt;F)</b>
Slope position	1.1258	1.1258	1	3.004	13.5885	0.03453 *
Fertilizer	13.8891	3.4723	4	274.516	41.9113	$<\!\!2  imes 10^{-16}$ ***
Genotype	0.5367	0.2684	2	274.036	3.2392	0.0407 *
Fertilizer:genotype	0.7399	0.0925	8	274.873	1.1163	0.3522

Significance codes: \*\*\* <0.001; \*< 0.05.

Genotypes differed significantly in both the yield (p < 0.001) and biomass (p < 0.05) of finger millet, showing the average result of Diga-01 > Meba > Urji. Similarly, fertilizer treatment significantly (p < 0.001) affected both the yield and biomass of finger millet, showing the average results of T5 > T1 > T3 > T2 > T4 and T3 > T1 > T2 > T5 > T4, respectively.

Strong evidence (p < 0.001) was seen for the fertilizer's effect on finger millet plant height as well as on finger length irrespective of location, slope and genotype. The genotype shows strong evidence (p < 0.001) for its effect on finger length and for some effect on plant height (p < 0.05). Slope position shows weak evidence (p < 0.1) for its effect on plant height.

#### 4. Discussion

The present study reports finger millet genotypic responses to Zn and Fe agronomic biofortification, location and slope position in yield and biomass. Irrespective of genotype, locations and slope positions, grain yield was enhanced by 20% due to the soil application of FeSO<sub>4</sub> fertilizer. However, different finger millet genotypes responded differently to both fertilizer treatment and location with respect to yield and yield traits. This suggests that

finger millet genotypes differ in their ability to remobilize and retranslocate deposited Zn and Fe, which plays a critical role in the improvement of the partitioning of carbohydrates from the leaves to the reproductive parts, affecting the yield and yield attributes. Therefore, the current finding should be taken into consideration in evaluating cereal genotypes for their responses to agronomic biofortification. To our knowledge, there is no available previous data on finger millet with Zn, Fe, or combined fertilizer, with genotype, location, and slope position effects, on grain yield and yield traits. Thus, this experiment is the first of its kind to report the triple impact of Zn and Fe agronomic biofortification, genotype, and environment (location and slope position) on the grain yield and biomass of finger millet. However, previous studies show that different finger millet genotypes responded differently to NPK fertilization in India [21], to phosphorus fertilizer in locations in Kenya [22], and to location in Ethiopia [23]. On the other hand, wheat and rice genotypes responded differently to Zn fertilization in Turkey [8] and to Zn fertilization as well as climate in India [24], respectively.

# 4.1. Finger Millet Genotypic Response to Zn Fertilizer towards Yield Affected by Location and Slope Position

The present study indicates that the Urji genotype responds significantly to ZnSO<sub>4</sub> fertilizers at the Gojjam hill slope position, where a 27.6% yield increase was observed. The possible reason behind the fact that finger millet responded well at the Gojjam hill slope position to Zn fertilization is that the soil sulphur concentration is significantly higher at the Gojjam hill slope position (Table 1), since sulphur is reported to enhance the solubility of Zn and its uptake by the plant [25]. On the other hand, the application of ZnSO<sub>4</sub> fertilizers significantly increased the total and productive tiller number for the Urji genotype at the hill slope position (Table 4), and this might have also played a major role in the yield increase. There are no available previous data that explore the impact of Zn fertilization on finger millet grain yield. However, previous research on wheat, rice, maize, sorghum, etc, has explored the effect of Zn fertilization on grain yield. A study from India showed a 14.2% yield increase as a result of the application of Zn fertilization [26]. Similarly, Phattarakul et al. [27] reported an increase of 10% in crop yield in their experiment, which was conducted in China and India. Another experiment from India indicated a 23.5% increase in grain yield by applying Zn fertilization [28]. An increase in yield of up to 33% was observed as a result of the application of Zn fertilizer [24]. Narwal et al. [29] also found a 5% yield enhancement by applying Zn fertilizer. Overwhelming evidence from all over the world indicates that the application of Zn fertilizer improves crop yields [1,2,8,30-45]. The positive response of yields in cereal to Zn fertilization in both current and previous studies is possibly due to the enhancement of plants' available Zn for uptake [8], which in turn helps the plants achieve better protein breakdown and enzyme activation, resulting in higher vegetative growth and yield increases [28]. However, one study from Thailand and Turkey shows little or insignificant effect of Zn fertilizer application on yield [27]. The irresponsiveness of rice to Zn fertilization in a previous study was possibly due to the absence of sulphur fertilization, since sulphur has been reported to enhance the solubility of Zn and its uptake by spring wheat [25]. In addition to that, a lower soil Zn concentration was reported (ranging from 0.5 to 6.5 mg kg $^{-1}$  in a previous study, in contrast to 85 to 105 mg kg $^{-1}$  in the present study). The other possibility might be that different crop genotypes responded differently to Zn fertilizer treatment, climate and environment (location and slope position), as was witnessed in the current as well as previous studies [8,23,24,45].

## 4.2. Finger Millet Genotypic Response to Fe Fertilizer towards Yield Affected by Location and Slope Position

The present study indicates that the Diga-01 genotype responded significantly to  $FeSO_4$  fertilizer application at the Arsi Negelle hill slope position, in which an 18.3% average yield enhancement was recorded. The possible reason behind the fact that finger millet responded well at the Arsi Negelle hill slope position to Fe fertilization is that the soil potassium concentration is significantly higher at the Arsi Negelle hill slope position

(Table 1), since it is reported that potassium seems to have a very specific role in the plant for the maximum utilization of Fe [46,47]. On the other hand, application of FeSO<sub>4</sub> fertilizers significantly increased the total and productive tiller number for the Diga-01 genotype at the hill slope position (Table 3), and this might also play a major role in yield increase. Even though no data are available for Fe fertilization's impact on finger millet grain yield, a few previous experiments on wheat, barley and oats have investigated the effect of Fe fertilization on grain yield and are discussed with the current result. The agronomic biofortification of Fe fertilizer is less well studied as compared to Zn fertilizer. For instance, a study from India indicated an enhancement of 13% in yield due to the application of Fe fertilizer [29]. The positive response of yield to Fe fertilization in both current and previous studies is possibly due to the enhancement of plant-available/soluble Fe for uptake [8], which in turn helps the plant achieve better chlorophyll synthesis, protein and carbohydrate metabolism and enzyme activation, resulting in better vegetative growth and yield increases [48]. However, studies from Turkey and Canada on Fe biofortification showed no yield improvement [30,49]. This might be due to three possible reasons: the first reason could be that, in general, different crops and genotypes responded differently to mineral fertilization as well as location [1,23,24]. The second possibility is that when applied to calcareous soils, Fe rapidly converted into unavailable forms, and the poor mobility of Fe in phloem makes the Fe fertilization impact limited or unsuccessful [2,44]. It is also possibly due to the graminaceous species' release of phytosiderophores (Fe-mobilizing compounds) to solubilize and absorb Fe from calcareous soils with low Fe concentrations; thus, they can maintain adequate plant growth by satisfying Fe demand without the requirement for Fe fertilization [50,51].

#### 5. Conclusions

Finger millet genotypes greatly influenced the response to agronomic biofortification of Zn and Fe fertilizer in the present study, which indicates the varied yield and yield traits performances of the genotypes across different environments (location and slope position). This reveals the vitality of experimenting on finger millet genotypes' responses to Zn and Fe fertilizer in different environments (location and slope positions) prior to a scale-up for mass production. The soil application of 20 kg of FeSO<sub>4</sub>7H<sub>2</sub>O per hectare along with the recommended rate of NPKS could be an excellent agronomic biofortification strategy to enhance the yields of all genotypes in the study areas and in areas with similar agro-ecologies. Moreover, the soil application of 20 kg ZnSO<sub>4</sub>7H<sub>2</sub>O and of a combined 20 kg FeSO<sub>4</sub>7H<sub>2</sub>O and 25 kg ZnSO<sub>4</sub>7H<sub>2</sub>O in Urji and Meba, respectively, at the Gojjam hill slope and in areas with similar agro-ecologies, could be a premium agronomic biofortification strategy to improve finger millet grain yield. Future studies as well as the development of programs on agronomic biofortification should consider environmental (location and slope position) effects in addition to the main fertilizer effect, which is a gap in the current knowledge.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061452/s1.

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