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Towards Zinc Biofortification in Chickpea: Performance of Chickpea Cultivars in Response to Soil Zinc Application

Legesse Hidoto [1,2], Bunyamin Tar'an [3,*], Walelign Worku [1] and Hussein Mohammed [1]

1 School of Plant and Horticultural Sciences, Hawassa University, P.O. Box 5, Hawassa, Ethiopia; hidotog@yahoo.com (L.H.); walelignworku@yahoo.co.uk (W.W.); aminaa68@yahoo.com (H.M.)
2 Hawassa Agricultural Research Center, P.O. Box 6, Hawassa, Ethiopia
3 Crop Development Centre/Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
* Correspondence: bunyamin.taran@usask.ca; Tel.: +1-306-966-2130; Fax: +1-306-966-5015

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Abstract: A field experiment was conducted at three locations in the southern region of Ethiopia during the 2012 and 2013 cropping seasons to evaluate chickpea cultivars for their response to soil zinc application, including agronomic performance, grain yield, grain zinc concentration, zinc and agronomic efficiency. Fifteen chickpea cultivars were evaluated in a randomized complete block design with three replications at each location and year. The highest number of pods (237) plant^{-1} was obtained from Butajira local landrace. The cultivar Naatolii produced the highest grain yield (2895 kg·ha^{-1}), while the breeding line FLIP03-53C had the lowest yield (1700 kg·ha^{-1}). The highest zinc concentrations of 47.5, 47.4, and 46.4 mg·kg^{-1} grain were obtained from the cultivar Arerti, and the two breeding lines FLIP07-27C and FLIP08-60C, respectively. The highest zinc efficiency (88%) was obtained from the Wolayita local landrace, whereas the highest agronomic efficiency of 68.4 kg yield increase kg^{-1} zinc application was obtained from the cultivar Naatolii. The current research identified chickpea cultivars with high grain zinc concentration, zinc efficiency, agronomic efficiency, and grain yield. The identification of cultivars with high grain zinc concentration allows the use of chickpea as a potential alternative to help to correct zinc deficiency, which is highly prevalent in the population of the region.

Keywords: chickpea; cultivars; zinc application; grain zinc concentration; biofortification

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume in the world ranking second after dry bean and constitutes 20% of the world's pulses production [1]. The crop plays an important role in human diet and agricultural systems [2]. Chickpea is high in protein, low in fat and sodium, cholesterol free, and is an excellent source of both soluble and insoluble fiber, complex carbohydrates, vitamins, folate, and minerals, especially calcium, phosphorus, iron, zinc, and magnesium [3–5].

Ethiopia is the largest producer of chickpea in Africa, accounting for 46% of the continent's chickpea production during 1994–2006 [6]. In 2011, Ethiopia produced 400,208 tons of chickpea from a total area of 231,300 hectares [7]. In 2012, the chickpea seeded area in Ethiopia increased by 8200 hectares from the previous year, totaling 239,500 hectares with the total production of about 409,733 tons [8]. The consistent increase in cultivated area and production implies the importance of the crop to the country.

Currently, there are major challenges to chickpea production in Ethiopia, such as soil nutrient deficiency of both macro and micronutrients, since the crop is often grown on marginal land, limited availability of improved cultivars, diseases, insects, and moisture stress. Chickpea, in particular, is considered highly sensitive to zinc deficiency, which is common in the major chickpea-growing regions of the world, including Ethiopia, and limits the crop productivity [9].

Wuehler et al. reported that approximately 20.5% of the world's population is estimated to be at risk of inadequate Zn intake, with the percentage of individuals at risk highest in the South East Asia (33%) followed by Sub Saharan Africa (28%), South Asia (27%), Latin America and the Caribbean (25%) [10]. In Ethiopia, micronutrient deficiency remains a significant public health concern, especially deficiencies in iron, vitamin A, folic acid, iodine, and zinc (Zn), affecting the physical and mental functioning and growth, brain development during pregnancy, visual impairment, increased susceptibility to diseases, and increased mortality risk [11]. The problem is more acute in southern Ethiopia where the diets are heavily dependent on cereals and root crops, which inherently are low in micronutrients and high in carbohydrates.

A number of studies have reported the growth and yield responses of chickpea cultivars grown under micronutrient deficient soils [12–16]. The magnitude of yield losses in chickpea due to nutrient deficiency varies among the nutrients [17]. The use of fertilizer can be considered an important complementary approach to the breeding efforts to develop cultivars adapted to soils with low micronutrient availability. A recent report by Shivay et al. [18] indicated that the application of Zn had a positive effect on grain yield and seed Zn concentration, especially under Zn deficient soils. However, such information for Ethiopian agro-climatic and diverse soil conditions and the currently available chickpea cultivars is not available.

Sadeghzadeh reported that plant cultivars vary in their tolerance to soils with low plant-available Zn with respect to Zn uptake and utilization [19]. Tolerance of plant cultivars to Zn deficiency is usually referred to as Zn efficiency and is defined as the ability of a cultivar to grow and yield well in soils that are deficient in Zn to support a given cultivar. The physiological and molecular mechanisms of tolerance to low Zn are just beginning to be understood, and these mechanisms can be exploited in crop improvement programs [20]. Cultivars with better Zn utilization may contain higher amounts of chelators that bind Zn and increase its physiological availability at the cellular level [21]. A better understanding of the response to zinc deficiency of different cultivars is needed for the development of fast, simple, and reliable screening procedures for identifying and breeding cultivars for Zn efficiency [22].

The experiment presented in this paper was conducted to examine the response to soil zinc application of chickpea cultivars available in the southern region of Ethiopia. Specifically, we evaluated the grain zinc concentration, zinc and agronomic efficiency, general agronomic performance, and yield potential of the released chickpea cultivars, newly introduced breeding lines, and local landraces in response to soil Zn application.

2. Results

The variations among cultivars, environments, and their interactions for most of the parameters were significant. However, grain zinc concentration was not affected by this interaction (Table 1).

Table 1. Analysis of variance table showing mean squares (MS) of the effect of ENV (site-year) and their interaction on plant height, biomass, pods plant^{-1}, seed weight, and grain zinc concentration across 15 chickpea cultivars and breeding lines (GEN).

Sources of Variation	*df*	Plant Height	Biomass	Pods Plant^{-1}	1000 Seed Weight	Grain Yield	Grain Zn
ENV	5	1082 **	543,137,609 **	84,752 **	6191 **	8000.8 **	2757 **
REP(ENV)	12	69	1,938,876	1708	183	24.8 *	50
GEN	14	911 **	2,826,672	3094 **	98,717 **	152.2	99 **
ENV × GEN	70	61 **	2,017,462 **	832 **	573 **	90.6 ***	30
ERROR	168	22	537,045	390	274	11.6	23
CV%		6.52	13.54	23.67	5.96	6.8%	10.89

* = significant at 5%, ** = significant at 1%, *df* = degrees of freedom, REP = replication, CV = coefficient of variation.

2.1. Plant Height

Significant differences in plant height were observed among cultivars across locations and years ranging from 48 cm for Wolayita local landrace at Huletegna Choroko in 2012 to 90 cm for the advanced breeding line (FLIP03-128C) at Jolle Andegna in 2013. In general, the advanced breeding lines were taller than either the released cultivars from the national program or the local landraces (Table 2).

Table 2. Plant height (cm) of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Wolayita local	73	77	66	70	48	51	64
Butajira local	70	73	67	71	50	53	64
Arerti	65	68	67	70	60	63	66
Cheffe	77	81	69	73	61	64	71
Ejeri	71	75	71	75	68	72	72
Habru	77	80	68	72	61	64	70
Mastewal	69	73	65	68	54	57	64
Naatolii	68	71	61	64	59	62	64
Shasho	66	70	73	77	63	66	69
FLIP03-53C	85	89	80	84	81	85	84
FLIP03-102C	76	80	74	78	79	83	78
FLIP03-128C	86	90	74	77	78	82	81
FLIP07-81C	80	84	74	79	69	73	77
FLIP08-60C	85	89	73	77	74	78	79
FLIP07-27C	85	89	71	75	81	85	81
Env. mean	76	79	70	74	66	69	72
SE (±)	3.4	3.6	2.01	2.12	2.39	2.47	1.11
$LSD_{0.05}$	9.77	10.40	7.15	6.15	6.92	5.82	3.11

SE = standard error, LSD = least significant difference.

2.2. Above Ground Biomass

There was a significant ($p < 0.05$) cultivar by environment interaction effect on the aboveground biomass of the chickpea plants. Mean aboveground biomass obtained at Jolle Andegna 2013, Taba 2013, and Huletegna Choroko 2013 was significantly higher than the biomass produced at the same locations in 2012. More than a fivefold biomass difference was observed between Jolle Andegna in 2013 and Jolle Andegna in 2012. The highest aboveground biomass was obtained from the FLIP03-128C breeding line, which had 36% more biomass than that obtained from the lowest cultivar Wolayita landrace (Table 3).

Table 3. Aboveground biomass ($t \cdot ha^{-1}$) of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Wolayita local	1.97	9.63	1.64	6.43	2.11	5.00	4.46
Butajira local	1.76	9.88	1.77	7.83	4.14	5.50	5.15
Arerti	1.37	10.50	2.51	9.40	2.83	6.50	5.52
Cheffe	1.31	12.13	2.58	8.27	3.05	6.50	5.64
Ejeri	1.97	9.95	2.70	8.42	1.89	6.23	5.19
Habru	1.70	9.83	1.94	8.03	2.20	6.50	5.03
Mastewal	2.65	9.87	2.77	7.73	2.87	5.85	5.29
Naatolii	1.99	9.47	2.00	7.60	3.45	6.32	5.14
Shasho	2.60	9.33	2.61	9.38	2.59	6.67	5.53
FLIP03-53C	1.95	9.73	2.79	9.47	3.21	7.63	5.80

Table 3. *Cont.*

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
FLIP03-102C	2.02	10.65	3.00	8.43	1.58	8.12	5.63
FLIP03-128C	3.26	10.70	1.89	9.63	2.83	8.13	6.07
FLIP07-81C	2.32	9.83	2.88	7.45	2.62	6.53	5.27
FLIP08-60C	1.40	11.80	1.66	8.23	2.54	8.43	5.68
FLIP07-27C	2.09	10.13	1.58	7.07	6.16	7.77	5.80
Env. Mean ($t{\cdot}ha^{-1}$)	2.02	10.23	2.29	8.22	2.94	6.78	5.41
SE (±)	0.21	0.64	0.35	0.54	0.23	0.39	0.17
$LSD_{0.05}$	0.61	NS	1.01	1.58	0.68	1.13	0.48

SE = standard error, LSD = least significant difference, NS = non-significant.

2.3. Number of Pods Plant^{-1}

There was a significant number of pods plant^{-1} variations among the cultivars across the environments. The Butajira landrace at Jolle Andegna in 2013 produced the largest number of pods (237) plant^{-1}, whereas cultivar Cheffe at Huletegna Choroko in 2012 produced the least (18) pods plant^{-1}. The difference between the highest and the lowest mean number of pods plant^{-1} for genotypes across the environments was 107%. In general, the local materials produced more pods plant^{-1} than the improved or advanced breeding lines across the environments (Table 4).

Table 4. Number of pods plant^{-1} of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Wolayita local	53	222	60	52	49	95	89
Butajira local	50	237	42	54	44	63	82
Arerti	38	161	39	49	34	73	66
Cheffe	21	122	38	37	18	61	50
Ejeri	45	158	30	41	38	53	61
Habru	32	144	38	39	29	71	59
Mastewal	47	188	44	39	44	56	70
Naatolii	43	119	41	36	40	45	54
Shasho	38	124	51	36	35	63	58
FLIP03-53C	26	130	25	28	26	53	48
FLIP03-102C	29	140	30	36	26	50	52
FLIP03-128C	34	146	23	55	31	57	58
FLIP07-81C	30	95	23	59	30	46	47
FLIP08-60C	33	87	26	39	33	56	46
FLIP07-27C	31	104	21	35	31	33	43
Env. mean	37	145	35	42	34	58	59
SE (±)	4.2	23.2	4.79	6.93	3.80	11.77	4.65
$LSD_{0.05}$	12	67	11	NS	14	NS	6.42

SE = standard error, LSD = least significant difference, NS = non-significant.

2.4. Thousand Seed Weight

Thousand seed weight was significantly affected by cultivar and environment interaction. The local materials had significantly lower seed weight than the released cultivars and advanced breeding lines across the environments (Table 5).

Table 5. Thousand seed weight (g) of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Wolayita local	104	112	119	127	111	119	115
Butajira local	108	116	111	119	113	121	115
Arerti	243	260	265	285	235	251	257
Cheffe	293	315	305	329	297	320	310
Ejeri	287	307	299	321	289	311	302
Habru	276	297	301	324	272	292	294
Mastewal	229	247	221	237	193	209	223
Naatolii	283	304	300	321	248	267	287
Shasho	273	293	281	303	243	260	276
FLIP03-53C	309	332	325	349	315	339	328
FLIP03-102C	329	353	329	353	355	381	350
FLIP03-128C	289	311	320	344	321	345	322
FLIP07-81C	324	348	328	352	328	352	339
FLIP08-60C	299	321	279	299	305	329	305
FLIP07-27C	325	349	313	336	349	376	341
Env. mean	265	284	273	293	265	285	278
SE (±)	9.1	9.8	11.28	12.03	6.70	7.23	3.90
$LSD_{0.05}$	26.3	28.4	32.7	34.8	19.4	20.9	10.89

SE = standard error, LSD = least significant difference.

2.5. Grain Yield

Results of a homogeneity of variance test revealed that environments were heterogeneous for grain yield. The grain yield data were then log transformed and used for combined analysis. The original yield data are presented in the results while the transformed values were included in parentheses. The grain yield of each cultivar obtained at each location in 2012 was significantly lower than that obtained in 2013 (Table 6). Overall, cultivar Naatolii produced the highest grain yield (2.9 $t \cdot ha^{-1}$) across all environments.

Table 6. Grain yield ($kg \cdot ha^{-1}$) of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012 (E1)	2013 (E2)	2012 (E3)	2013 (E4)	2012 (E5)	2013 (E6)	
Wolayita local	783 (36)	5600 (70)	1103 (41)	3433 (62)	1013 (41)	2667 (57)	2433 (51)
Butajira local	697 (34)	5800 (71)	1070 (41)	3600 (63)	2110 (53)	2500 (56)	2630 (53)
Arerti	493 (28)	6067 (72)	1230 (44)	3133 (60)	1680 (49)	3100 (60)	2617 (52)
Cheffe	183 (11)	5667 (70)	1483 (47)	2800 (58)	2040 (53)	2900 (58)	2512 (50)
Ejeri	683 (34)	5533 (69)	1407 (46)	3400 (62)	1013 (41)	3033 (60)	2512 (52)
Habru	590 (31)	5100 (69)	1187 (43)	2400 (55)	1547 (48)	3167 (60)	2332 (51)
Mastewal	713 (34)	5733 (71)	1677 (49)	2333 (55)	1620 (49)	3267 (61)	2557 (53)
Naatolii	660 (33)	7267 (75)	1173 (42)	2467 (56)	2470 (56)	3333 (61)	2895 (54)
Shasho	870 (38)	4500 (66)	1207 (43)	2800 (62)	2070 (53)	2933 (58)	2397 (53)
FLIP03-53C	583 (31)	3467 (62)	1060 (41)	1800 (58)	622 (32)	2667 (58)	1700 (47)
FLIP03-102C	470 (27)	4867 (68)	1440 (47)	1833 (55)	577 (31)	2367 (55)	1926 (46)
FLIP03-128C	860 (38)	4800 (67)	857 (37)	2300 (54)	780 (36)	2133 (54)	1955 (48)
FLIP07-81C	913 (39)	5200 (69)	1567 (47)	2700 (58)	653 (33)	2767 (58)	2300 (50)
FLIP08-60C	673 (33)	4367 (66)	873 (38)	2167 (58)	670 (33)	2833 (58)	1933 (47)
FLIP07-27C	703 (34)	4033 (64)	917 (38)	2500 (54)	300 (19)	2233 (54)	1751 (44)
Environment mean	658 (33)	5200 (69)	1217 (43)	2644 (57)	1278 (42)	2793 (58)	2298 (50)
SE (±)	57.2 (1.52)	515.5 (1.92)	180.39 (2.76)	356.57 (2.42)	65.72 (0.96)	262.92 (1.66)	118.1 (2.24)
$LSD_{0.05}$	(4.14)	(5.57)	(NS)	(7.00)	(2.79)	(NS)	(NS)

SE = standard error, LSD = least significant difference, NS = non-significant; Numbers in parentheses refer to average data obtained from the analysis of variance of log-transformed data.

2.6. Grain Zinc Concentration

Main effects of cultivar and environment were significant on grain zinc concentration. However, no significant interaction effect of environments and cultivars was observed (Table 1). Significant variation in grain zinc concentration was observed among the cultivars. The cultivar Arerti and the two breeding lines (FLIP07-27C and FLIP08-60C) had the highest grain zinc concentration compared to the rest of the cultivars and breeding lines (Table 7). There was no significant difference in seed zinc concentration among the chickpea groups.

Table 7. Grain zinc concentration (mg·kg^{-1}), zinc efficiency (ZnE), and agronomic efficiency (AE) of 15 chickpea cultivars and breeding lines tested with (+Zn) and without zinc (−Zn) fertilizer application over the locations in 2012 and 2013.

Cultivars	Grain Zinc (−Zn) (mg·kg^{-1})	Grain Zinc (+Zn) (mg·kg^{-1})	Yld (−Zn) (t·ha^{-1})	Yld (+Zn) (t·ha^{-1})	ZnE (%)	AE
Wolayita local	36.7	43.6	2.14	2.43	88.1	11.5
Butajira local	37.2	45.4	1.90	2.63	72.3	29.1
Arerti	42.3	47.5	2.13	2.62	81.4	19.5
Cheffe	39.2	43.8	1.31	2.51	52.0	48.2
Ejeri	36.2	40.3	1.55	2.51	61.6	38.6
Habru	37.4	41.5	1.55	2.33	66.3	31.5
Mastewal	37.0	39.9	1.45	2.56	56.6	44.4
Naatolii	36.3	42.4	1.19	2.90	41.0	68.4
Shasho	38.1	42.8	1.27	2.40	53.1	45.0
FLIP03-53C	38.9	43.6	1.11	1.70	65.0	23.6
FLIP03-102C	40.2	45.1	1.04	1.93	53.8	35.6
FLIP03-128C	39.3	43.9	1.34	1.96	68.3	24.8
FLIP07-81C	37.4	41.8	0.77	2.30	33.4	61.3
FLIP08-60C	41.6	46.4	1.10	1.93	57.1	33.1
FLIP07-27C	42.5	47.4	1.45	1.75	81.4	13.2
Mean	38.7	43.7	1.42	2.30	61.8	35.5
SE (±)	0.55	1.12	0.10	0.04	3.88	4.22
LSD	1.55	NS	0.06	NS	-	-

Yld (−Zn) (ton·ha^{-1}) = yield obtained from no zinc fertilization; Yld (+Zn) (ton·ha^{-1}) = yield obtained from zinc fertilization; ZnE (%) = zinc efficiency = (yld (−Zn)/yld (+Zn)) × 100; AE = agronomic efficiency = (yld (+Zn)) − (yld (−Zn))/zinc supplied; SE is standard error of the mean; Note: 25 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1} was applied uniformly for all zinc amended plots. SE = standard error, LSD = least significant difference, NS = non-significant.

Grain zinc concentrations obtained from both Jolle Andegna 2012 and 2013 were significantly lower than the rest of the environments. For instance, zinc concentration values obtained from Jolle Andegna in both years were lower by 17% and 43% than Taba 2012 and Taba 2013, respectively (Table 8).

Table 8. Grain zinc concentration (mg·kg^{-1}) of 15 chickpea cultivars and breeding lines tested under zinc fertilized soil across three locations in southern Ethiopia during the 2012 and 2013 growing seasons.

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Wolayita local	36.53	42.63	30.83	51.10	51.70	48.67	43.58
Butajira local	36.10	32.47	42.87	51.30	58.63	50.77	45.36
Arerti	38.77	34.63	44.83	55.30	58.03	53.70	47.54
Cheffe	35.00	38.77	41.93	49.23	52.03	45.57	43.76
Ejeri	33.77	29.53	35.27	45.97	50.60	46.67	40.30
Habru	34.20	30.73	33.23	50.30	50.73	50.03	41.54
Mastewal	31.57	32.40	37.87	46.67	44.67	46.20	39.89

Table 8. *Cont.*

Cultivars	Jolle Andegna		Taba		Huletegna Choroko		Cultivar Mean
	2012	2013	2012	2013	2012	2013	
Naatolii	34.20	28.60	42.73	51.50	48.23	49.03	42.38
Shasho	34.27	30.90	39.93	50.10	54.23	47.43	42.81
FLIP03-53C	35.70	31.47	39.50	51.53	54.93	48.60	43.62
FLIP03-102C	34.80	32.33	45.33	49.37	55.03	53.53	45.07
FLIP03-128C	36.43	38.43	43.10	50.30	52.03	45.20	44.25
FLIP07-81C	32.23	36.07	45.03	45.07	47.67	42.47	41.42
FLIP08-60C	38.10	37.70	45.73	49.97	55.07	52.07	46.44
FLIP07-27C	35.90	44.33	45.93	53.43	58.13	46.63	47.39
Env mean	35.17	34.73	40.94	50.08	52.78	48.44	43.69
SE (±)	1.3	2.1	5.17	2.12	1.83	2.11	1.12
LSD5%	3.90	6.06	NS	NS	5.30	6.12	NS

SE = standard error, LSD = least significant difference, NS = non-significant.

2.7. Zinc and Agronomic Efficiency

Zinc efficiency is defined as the ability of a plant to grow and yield well under zinc-deficient conditions. As previously presented in Table 7, zinc efficiency varied among the cultivars and breeding lines. The Wolayita landrace had the highest zinc efficiency (88%) followed by the cultivar Arerti and the breeding line FLIP07-27C with 81% each. The lowest zinc efficiency of 33% was obtained from the FLIP07-81C breeding line. Cultivar Naatolii had the highest agronomic efficiency of 68.4 kg yield increase per kg zinc application, whereas the Wolayita landrace only responded by 11.5 kg yield increase for every kg zinc application.

3. Discussion

There was a highly significant ($p < 0.001$) difference among environments (site-year) for all the parameters. For instance, grain yield at Jolle Andegna 2013, Taba 2013, and Huletegna Choroko 2013 was 689%, 111%, and 116% greater than Jolle Andegna 2012, Taba 2012, and Huletegna Choroko 2012, respectively. This huge variation in grain yield among environments was attributed mostly to weather variation between the two years (2012 and 2013). Total precipitation during the growing season (August–December) in 2013 was much higher than that of 2012 at each location. For instance, the total rainfall for the growing season at Huletegna Choroko in 2013 was greater by 285 mm than that of 2012 at the same location. Rainfall in October and November at Jolle Andegna in 2012 was 15.8 mm and 8.8 mm, respectively. Consequently, plants were stressed and forced to mature with most of the pods aborted. Besides the amount of rainfall, its distribution during 2012 crop growing period was uneven with most of the days without rain and some days with less than 5 mm precipitation. Despite the large environmental effect, there were highly significant ($p < 0.01$) differences among the cultivars across environments (site-year) for grain yield and yield components. The newly introduced chickpea breeding lines had the highest plant height and aboveground biomass than either the local or the released cultivars. The highest number of pods plant^{-1} was obtained from the landraces; however, these did not translate into grain yield. The mean grain yield across environments indicated that cultivar Naatolii produced the highest grain yield (2.90 t·ha^{-1}), which was 71% higher than the yield of the FLIP03-53C (1.70 t·ha^{-1}) breeding line. The significant differences among cultivars attributed to the variation in the genetic potential of the cultivars in response to the stress and local environmental conditions. Some cultivars produced relatively better yield in harsh environments while others did not perform well, possibly due to their physiological and genetic makeup.

Identifying and promoting the use of chickpea cultivars with high seed Zn concentration is one strategy for improving human nutrition while increasing protein intake associated with consumption of the product. There was a highly significant variation in grain zinc concentration among the cultivars observed in the present study. The cultivar Arerti and the two breeding lines (FLIP07-27C and FLIP08-60C) had the highest seed zinc concentration compared to the rest of the cultivars and breeding lines. Several authors reported that there are significant variations in seed zinc concentration among chickpea cultivars [4,23–25]. The variation in seed zinc concentration of the current chickpea cultivars and breeding lines could be due to variation in seed physiology, morphology, and tissue zinc distribution, which all are under genetic control [26,27]. The variation in grain zinc concentration among the environments was also significant in the present study. The variation in grain Zn concentration across environments was due to the amount and distribution of rainfall. Moisture stress occurred at vegetative and pod setting stage may have reduced zinc absorption and accumulation in the seeds, as shown across locations in 2012; while relatively sufficient moisture was available in 2013, resulting in higher grain yield, but dilution of grain zinc by grain carbohydrate increments. Previously, significant environment variation in grain zinc concentration was reported [4,28]. When the soil remains wet and becomes reduced, the availability of Mn, Fe, Cu, and P usually increases, and this condition is reversed under dry soil conditions [29].

The variation in zinc efficiency between the highest and the lowest cultivars was about 167%. The physiological basis for Zn efficiency and its importance for plant adaptation under low soil Zn availability have been reported by several authors [30–32]. The genotypic differences in Zn efficiency may be associated with different mechanisms in rhizosphere and within a plant system. These included higher uptake of zinc by roots and efficient use and re-translocation of Zn [31]. Cakmak et al. [33] reported that Zn efficiency in cereal is mainly related to the difference in the acquisition of Zn by the roots. Graham and Rengel [34] reported that plant species vary significantly in response to micronutrient deficiency; some are able to cope with low micronutrient availability, and thus, grow well even when other species or cultivars suffer from reduced yield due to micronutrient deficiency.

Our results demonstrated that chickpea is a rich source of zinc (3.99–4.75 mg 100 g^{-1}). Similar results were observed previously [35]. Serving 42 g grain seeds of chickpea cultivars Arerti and FLIP07-27C (47.5 and 47.4 mg zinc kg^{-1} seed, respectively) could provide adequate zinc for infants 0–6 months; 63 g for 7 months–3 years; 105 g for 4–8 years; 168 g for 8–13 years; 232 g for 14+ years male and 19+ years pregnant; 253 g for 14–18 years pregnant and 19+ years lactating mother; and 274 g for 14–18 years lactating mother [36]. Thus, a single serving of zinc enriched chickpea could provide a marked amount of the recommended daily allowance (RDA) of zinc. Identification of cultivars with better zinc concentration like Arerti and FLIP07-27C may enable the use of chickpea as a potential whole food solution to micronutrient malnutrition in Ethiopia.

4. Materials and Methods

4.1. Description of the Study Area

The experiment was conducted at three locations (Huletegna Choroko, Jolle Andegna, and Taba) in the Southern Nations Nationalities and Peoples Region of Ethiopia during the growing seasons from August to December of 2012 and 2013. The altitude of the test locations ranges from 1807 to 1923 meters above sea level (m a.s.l.), annual rainfall ranged from 774 mm to 989 mm (which is ideal for chickpea production). The amount of rainfall in the 2012 growing season was much lower than that in the 2013 growing season, especially in October and November when the plants were at flowering and pod filling stages, respectively (Table 9).

Table 9. Geographic coordinates, altitude (meters above sea level, m a.s.l.), monthly rainfall (mm), and temperature (maximum, minimum, and mean) data during the 2012 and 2013 growing seasons for each research location.

Location	Geographic Coordinates	Altitude (m a.s.l.)	Months	Rain Fall (mm)		Temperature (°C)					
						Maximum		Minimum		Mean	
				2012	2013	2012	2013	2012	2013	2012	2013
Taba	7°01′01.9″ N and 37°53′57.6″ E	1915	August	169.7	223.0	21.5	21.5	13.5	13.4	17.5	17.5
			September	135.3	210.0	22.7	23.2	13.6	13.6	18.2	18.4
			October	13.0	150.0	26.2	24.2	13.5	13.6	19.9	18.9
			November	32.6	39.0	26.9	25.9	13.2	13.1	20.1	19.5
			December	16.8	16.0	27.3	26.5	12.8	12.6	20.1	19.6
Jolle Andegna	8°12′25.9″ N and 38°28′33.2″ E	1923	August	143.9	164.7	22.6	23.0	10.2	11.0	16.4	17.0
			September	88.0	48.1	24.1	28.4	9.9	11.2	17.0	19.8
			October	15.8	50.1	27.0	26.8	9.8	10.0	18.4	18.4
			November	8.8	33.5	27.0	26.8	9.1	9.5	18.1	18.2
			December	3.5	2.3	26.5	26.4	8.5	8.2	17.5	17.3
Huletegna Choroko	7°20′34.5″ N and 38°06′30″ E	1807	August	89.5	178.1	25.3	24.9	14.4	17.6	19.9	21.3
			September	100.7	138.8	27.1	28.2	14.1	17.4	20.6	22.8
			October	10.0	118.9	30.3	29.0	11.8	16.7	21.1	22.9
			November	9.0	60.3	31.7	30.2	12.0	11.8	21.9	21.0
			December	2.6	0.2	31.0	30.0	12.5	12.3	21.8	21.2

Source: National Meteorological Agency [37], Southern Zone, Hawassa Branch, Ethiopia.

4.2. Soil Analysis

Before sowing, composite soil samples were randomly collected across the research area using auger from the depth of 0–30 cm. The collected soil samples were air dried, cleaned of any stones and plant residues, and ground to pass a 2 mm sieve for the required analyses, including soil pH, electro conductivity (EC), organic carbon (OC), available P, total N, and Zn concentration. Soil organic carbon was determined by the Walkley procedure [38]. Soil P was extracted with NaHCO [39]. Soil Zn concentration was extracted with DTPA (diethylene triaminepenta acetic acid) and determined by AAS (Atomic Absorption Spectrophotometer) [40]. Analyses were conducted at the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University, Hawassa, Ethiopia.

Soils were considered to have low Zn availability when there was less than 1.1 mg Zn kg^{-1} soil following DTPA extraction [41]. For chickpea, the critical Zn concentrations in soils vary from 0.48 $mg{\cdot}kg^{-1}$ to 2.5 $mg{\cdot}kg^{-1}$, depending on soil type [9]. The DTPA extracted zinc concentration in the soils of the research sites ranged from 0.13 $mg{\cdot}kg^{-1}$ at Taba in 2012 to 0.98 $mg{\cdot}kg^{-1}$ at Huletegna Choroko in 2012, indicating that the soils were deficient in zinc (Table 10).

Table 10. Soil properties of the experimental sites at each location in 2012 and 2013.

Location	Year	pH (H_2O)	EC (ds/m)	Zn ($mg{\cdot}kg^{-1}$)	OC (%)	Total *N* (%)	Available P ($mg{\cdot}kg^{-1}$)	Soil Texture
Taba	2012	6.36	0.05	0.13	0.99	0.71	36.5	Silty loam
	2013	6.4	0.07	0.16	1.1	0.83	35.6	
Jolle Andegna	2012	6.77	0.2	0.17	1.71	0.57	27.1	Silty clay loam
	2013	6.82	0.22	0.19	1.7	0.65	30.2	
Huletegna Choroko	2012	6.73	0.08	0.98	1.78	0.44	37.6	Clay loam
	2013	6.93	0.09	0.94	1.73	0.46	38	

EC: electro conductivity; OC: organic carbon.

4.3. Plant Materials

Fifteen chickpea cultivars (seven released cultivars, six breeding lines, and two landraces) were used for the study. Seeds of the released cultivars were obtained from the Agronomy Section of the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University. The advanced breeding lines were obtained from ICARDA (International Center for Agricultural Research in the Dry Areas) through Debre Zeit Agricultural Research Institute, Ethiopia. Seeds of the local materials (land races) were obtained from the farmers in the local vicinity of the study areas. The description of the cultivars is provided in Table 11.

Table 11. Description of chickpea cultivars tested for their response to soil applied zinc fertilizer.

No.	Name	Type	Tested Line Number	Status
1	Wolayita local	Desi	Not available	Landrace
2	Butajira local	Desi	Not available	Landrace
3	Arerti	Kabuli	FLIP89-84C	Cultivar released in 1999
4	Cheffe	Kabuli	ICCV92318	Cultivar released in 2004
5	Ejeri	Kabuli	FLIP97-263C	Cultivar released in 2005
6	Habru	Kabuli	FLIP88-42C	Cultivar released in 2004
7	Mastewal	Desi	ICCV92006	Cultivar released in 2006
8	Naatolii	Desi	ICCX910112-6	Cultivar released in 2007
9	Shasho	Kabuli	ICCV93512	Cultivar released in 1999
10	FLIP03-53C	Kabuli	FLIP03-53C	Breeding line
11	FLIP03-102C	Kabuli	FLIP03-102C	Breeding line
12	FLIP03-128C	Kabuli	FLIP03-128C	Breeding line
13	FLIP07-81C	Kabuli	FLIP07-81C	Breeding line
14	FLIP08-60C	Kabuli	FLIP08-60C	Breeding line
15	FLIP07-27C	Kabuli	FLIP07-27C	Breeding line

Source: Released materials adopted from Ministry of Agriculture [6].

4.4. Design of the Experiment and Trial Management

A randomized complete block design (RCBD) with three replications was used for the experiment at each location and year. Plot size was 11.2 m^2, consisting of eight rows that were 3.5 m long each (Figure 1). Inter and intra-row spacing was 40 cm and 10 cm, respectively, resulting in 35 plants per row and 280 plants per plot. Di-ammonium phosphate (DAP) (18:46:0; N:P:K) at the rate of 60 $kg \cdot ha^{-1}$ was uniformly applied followed by zinc fertilizer ($ZnSO_4 \cdot 7H_2O$) at 25 $kg \cdot ha^{-1}$ drilled in rows and incorporated into the soil before chickpea sowing. Similarly, these cultivars (Table 11) were evaluated separately with no zinc fertilization on satellite plots at each location in the 2013 cropping season for evaluation of grain yield, zinc concentration, and zinc and agronomic efficiency. The experiments were planted at different dates across the locations and years based on the rainfall pattern and soil moisture content. At Huletegna Choroko the experiment was planted on 8 September 2012 and 23 August 2013. At Jolle Andegna, planting was done on 20 September 2012 and 4 September 2013, while at Taba planting was done on 14 September 2012 and 17 September 2013, respectively. Chickpeas are slow to emerge and initially grow slowly. They are notoriously poor competitors with weeds. Even moderate weed infestation can result in severe yield losses. Therefore, the plots were thoroughly and frequently weeded by hoeing and hand pulling when required. Herbicide was not used to control weeds. There was no serious problem of diseases or insects across the locations in both years.

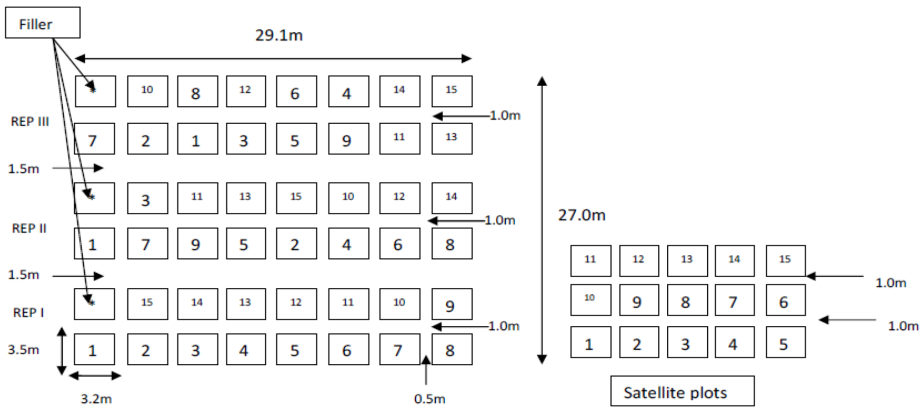

Figure 1. Schematic diagram showing treatment randomization and the experiment set up of satellite plots at one location. The same set up was used for each location and year. Plot sizes are the same for both experimental plots and satellite plots. Note: 1 = Arerti; 2 = Butajira Local; 3 = Cheffe; 4 = Ejeri; 5 = Habru; 6 = Mastewal; 7 = Naatolii; 8 = Shasho; 9 = Wolayita Local; 10 = FLIP03-53C; 11 = FLIP03-102C; 12 = FLIP03-128C; 13 = FLIP07-81C; 14 = FLIP08-60C; 15 = FLIP07-27C.

4.5. Agronomic Data Collection

Plant height and number of pods $plant^{-1}$ were recorded from 10 randomly selected plants from the middle six rows of each plot. Seed weight was determined by randomly selecting 250 seeds (10% moisture content) then weighed with a digital balance sensitive to the nearest 0.001 g. The value was then converted to 1000 seeds weight. Above ground biomass and grain yield were measured from the harvested plants of the middle six rows at maturity. The grain yield per plot was adjusted to storage moisture content (10%) determined using a digital grain moisture tester (HOH-EXPRESS HE 50).

4.6. Grain Analysis for Zinc Concentration

Grain zinc analysis was done at the College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Canada. Subsamples of seed (25 g) for measurement of zinc concentration were taken from each plot at each location and year. The samples were dried in an oven at 70 °C for 24 h, and ground using a sample rotating mill. A ground sample of 0.5 g was weighed on a balance sensitive to the nearest 0.00001 g and put into a digestion tube. The samples were prepared by a standard

HNO_3–H_2O_2 digestion method using wet digestion with nitric acid [42]. The Zn concentration was measured using flame AAS (AJ ANOVA 300, Lab Synergy). Zinc concentrations measured by this method were validated using the National Institute of Standards and Technology (NIST) reference material 1573a. Lentil (*Lens culinaris*) seeds (cv. CDC Redberry) and wheat (*Triticum aestivum* L.) were used as laboratory reference materials and measured periodically to ensure consistency in the procedure.

Zinc efficiency (ZnE) and agronomic efficiency (AE) were calculated following [43] as follows:

$$\mathrm{ZE} = \left(\frac{\mathrm{YdZn}^-}{\mathrm{YdZn}^+}\right) \times 100 \quad (1)$$

where ZE is Zinc efficiency, YdZn^- is grain yield in no Zinc supplied, and YdZn^+ is grain from zinc fertilized plots;

$$\mathrm{AE} = \frac{(\mathrm{YdZn}^+) - (\mathrm{YdZn}^-)}{\mathrm{ZnS}} \quad (2)$$

where AE is Agronomic efficiency, YdZn^+ is grain yield from Zinc fertilized plots; YdZn^- is grain yield from no Zinc application, and ZnS is supplied Zinc in $\mathrm{kg \cdot ha^{-1}}$.

4.7. Statistical Analysis

Each location-year combination was considered as a separate environment in this study, producing six environments (E1–E6) which were considered random. The General Linear Model (GLM) of the SAS software [44] was used for ANOVA of data from individual locations and for the combined data. Prior to the combined ANOVA, homogeneity of error variances over the six environments was tested. Mean separation was done using Least Significant Difference (LSD) test at the 5% level.

5. Conclusions

Chickpea cultivars evaluated in this study differed in grain zinc concentration, agronomic efficiency, zinc efficiency, growth, and yield. The cultivar with high yield and highest agronomic efficiency (Naatolii), the cultivar and breeding lines with better grain zinc concentration (Arerti, FLIP07-27C, and FLIP08-60C), and the cultivars with higher zinc efficiency (Wolayita landrace and breeding line FLIP07-27C) were identified. Serving chickpea grain seeds from the genotypes identified for their higher zinc concentrations could provide a marked amount of the recommended daily allowance (RDA) of zinc for infants, children, and pregnant and lactating mothers. This may enable development of chickpea-based whole food solutions to correct zinc malnutrition. Zn fertilization can also be blended with other Zn-containing fertilizer elements to reduce expenditure in terms of labor and time. Thus, this study provided a possibility for zinc biofortification through screening chickpea cultivars, which could be an attractive option for resource poor farmers across Ethiopia who cannot afford fortified foods or animal sources for their zinc nutritional requirement.

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