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Reducing anti-nutritional factor and enhancing yield with advancing time of planting and zinc application in grasspea in Ethiopia

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Abstract

BACKGROUND: Grasspea (*Lathyrus sativus* L.) is an important pulse crop for food, feed and sustainable crop production systems in Ethiopia. Despite its advantages in nutrition and adaptability to harsh climate and low fertile soil, it contains a neurotoxin, β-*N*-oxalyl-α,β-diamiono propionic acid (β-ODAP), which paralyses the lower limbs and is affected by genotypic and agronomic factors. To determine the effect of zinc application and planting date on yield and β-ODAP content of two genotypes, experiments were conducted in two regions of Ethiopia.

RESULTS: The main effects of variety, sowing date and zinc and their interactions were significant ($P < 0.001$) for β-ODAP and seed yield, which had a linear relationship with zinc. For the improved grasspea variety, an application of 20 kg ha^{-1} zinc showed a reduction of β-ODAP from 0.15% to 0.088% at Debre Zeit and 0.14% to 0.08% at Sheno and increased its yield from 841 kg ha^{-1} to 2260 kg ha^{-1} at Debre Zeit and from 715 to 1835 kg ha^{-1} at Sheno. Early sowing showed a reduction in ODAP content in relation to the late sowing.

CONCLUSION: An application of Zn beyond even 20 kg ha^{-1} with an early sowing is recommended for the improved variety.
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Keywords: grasspea; β-ODAP; zinc application; planting date; Ethiopia

INTRODUCTION

Grasspea (*Lathyrus sativus* L.) is an important pulse crop for food, feed and sustainable crop production system. It is rich in protein and many essential micro-nutrients, contributes to nutrition sustainability of the poor in Bangladesh, India, Ethiopia, Nepal, Eritrea and parts of China, and forms a significant construct in diversifying the cropping systems.[1,2] It is grown in about 1.1 million hectares globally of which about 0.16 million hectares is in Ethiopia. Its delicious and the soft fodder supplies quality roughage requirements for livestock; its straw is valuable in animal feed as well.[3] Due to its ability to fix nitrogen, like other pulse crops, grasspea adds substantial quantities of nitrogen, carbon and organic matter deposition in soil; the succeeding crop needs less fertiliser input, thus reducing the cost of cultivation. It is a very hardy crop with respect to drought tolerance, fewer attacks by pests and diseases and can also thrive well under waterlogging conditions. When other crops fail due to drought and adverse environmental conditions, it serves as a human staple food in times of famine. Grasspea is known by various local names such as ‘Khesari’ in Bangladesh and eastern India and Nepal, ‘Tiwara’ and ‘Latki’ in central India, ‘Guaya’ in Ethiopia and Eritrea, ‘San li dow’ in China, and ‘Mürdümük’ in Turkey. Grasspea is generally consumed by tribal people and poor sections of the society which cannot afford other costly pulses and mineral products.

Despite its multifarious agronomic, ecological and nutritional advantages, grasspea has an ambivalent reputation. Its plant-parts contain a neurotoxin, β-*N*-oxalyl α,β-diamiono propionic acid (β-ODAP), which is a neurotoxic non-protein amino acid that causes irreversible paralytic symptoms of the lower limbs/legs if consumed in large quantities over a long period of time. Outbreaks of this disease were evident in Ethiopia, Bangladesh, India and China at various periods of food scarcity.[2,4–9] Grasspea is mostly consumed through green parts, particularly tender twigs used as leafy vegetables, but its seed is the main component used for human food as well as various preparations. Its split cotyledons are used as ‘Dal’ in South Asia and eaten with rice/bread and as ‘Oat’ in Ethiopia. Its flour is also used for making various palatable snacks like ‘Piaju’ in Bangladesh and ‘Pakoura’ in India. Indian consumers found that ‘Pakoura’ made of a mix of chickpea and grasspea flour gives more palatability or enhanced taste. Based on a review of the effects of different processing methods on nutritional composition and anti-nutritional factors with a view to improve the nutritional quality and reducing the anti-nutritional factors of grasspea,

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the optimisation of processing conditions to minimise the nutrient loss has been recommended.[10]

Some researchers have reported an association between β-ODAP content and seed coat colour. Genotypes with white seed coat have low β-ODAP contents. Moreover, when the seeds are boiled, the effect of β-ODAP is reduced. But caution has been recommended for consuming seed and forage of grasspea, and grasspea products only in small quantities in order to avoid the neuro-lathyrism disorder.[11–13] β-ODAP concentration in grasspea seeds is reported to have high genotype × environment (G × E) interaction. To ensure safe consumption of grasspea with lower β-ODAP concentration in food products, there are various options such as genetic detoxification through breeding, agronomic manipulation and physico-chemical detoxification. Of these, we have undertaken agronomic means to lower β-ODAP concentration in this study. Among many environmental and edaphic factors, drought, salinity, and zinc concentration in soil have direct influence on seed β-ODAP concentration. For example, a crop grown under drought conditions and under zinc deficient soil will have more β-ODAP content in seeds.[9,14–16] In an extensive evaluation of grasspea germplasm of different origins during 1989–1990 with a view to identify toxin-free lines, several lines had low β-ODAP content but no line was β-ODAP free.[17] However, a number of cultivars with low toxin content compared to local landraces were found in Ethiopia.[18] The effect of zinc and phosphorus application on β-ODAP and yield of grasspea were evaluated in northern Syria,[15] while in another study, a number of improved lines were found to contain <0.10% β-ODAP and the factors such as zinc application, early sowing and seed soaking had a favourable effect on the toxin reduction.[19]

The International Center for Research in the Dry Areas (ICARDA) is working on grasspea to develop low β-ODAP varieties and production technologies which will ensure low β-ODAP synthesis in its seeds and to deliver these improved technologies to the national programmes of Ethiopia, among other grasspea growing countries. In this study, an experiment with an improved promising line and a local variety in two representative grasspea growing regions in Ethiopia, was conducted to evaluate (1) the effect of zinc application and planting date on yield and β-ODAP content of grasspea varieties and their interaction, and (2) modelling of β-ODAP content and yield in terms of zinc application to the soil to compare the above factors.

MATERIAL AND METHODS

Experiments were conducted for 2 years (2001/2002 and 2002/2003) at two locations, Debre Zeit and Sheno, where the soil was low in available zinc at < 0.4 mg kg^{-1} (Debre Zeit) and 0.3 mg kg^{-1} (Sheno). Debre Zeit Agricultural Research station (latitude: 10° 34′ 60 N; longitude: 35° 47′ 60 E, altitude: 2539 m asl) in central Ethiopia has a deep heavy, black clay (vertisol), with a long-term (1953–2003) average rainfall of 839 mm and temperature of 18°C. Its agroecology is tepid to cool sub-moist with dry (October to May) and rainy (June to September) seasons.[16] Sheno (latitude: 9° 20′ N; longitude: 39° 18′ E, altitude: 2800 m asl) has about 60% clay and an annual rainfall of about 900 mm, with excess rain in July and August (http://www.fao.org/wairdocs/ilri/x5493e/x5493e0w.htm).

At each of these two locations, an split-plot experiment in randomised complete blocks with three replications was conducted, where the two sowing dates, 29 August and 12 September (during 2001 and 2002), were allotted to the main plots, and factorial combinations of two genotypes ILAT-LS-520 and the Debre Zeit local landrace of dark seed coat colour and the five levels of zinc doses (0, 5, 10, 15, 20 kg ha^{-1}) in the form of basic zinc sulfate ($ZnSO_4$) were applied to the sub-plots. The sub-plot size was 2 m × 4 m and the spacing between main plots was 1.5 m. The β-ODAP content was determined following the standard calorimetric method of Rao[20] which was further modified by Briggs *et al.*[21] and seed yield was recorded on a per plot basis.

Statistical analysis

Analysis of variance (ANOVA) was carried out to assess the effect of sowing date (SD), genotypes (G) and the zinc applied, and their interactions, as well as interactions of these effects with location and year on β-ODAP content and seed yield. The location and year combination was partitioned as location and year within locations since the year-to-year climatic condition may not be assumed to be the same over each location. Furthermore, the location effects were assumed as fixed and year effects as random.[22] The ANOVA structures of the standard split-plot design was considered for individual location and year combination, while a combined analysis was carried out over location and years. It was observed that the error variances for β-ODAP content were homogeneous over the year–location combinations, therefore pooled ANOVA with a common error variance was carried out. For seed yield, the error variances were heterogeneous, so weighted ANOVA was carried out on the combined data to examine significance of main effects and interactions. Noticing the fact that most of the interactions with year were not significant but with locations were significant ($P < 0.001$), we carried out separate unweighted ANOVA for the split-plot design data combined over the years for each location, and estimated means with standard errors for comparisons. Furthermore, the variation due to zinc was partitioned into polynomial components to guide on β-ODAP content and zinc dose relationship. The mean response on β-ODAP content and yield were modelled using linear functions of applied zinc dose for each combination of genotype, planting time and locations. All the computations were carried out using GenStat statistical software (VSN International, 2015).[23]

RESULTS

Statistical analysis of β-ODAP content and seed yield of grasspea was carried out to assess the main effects and interactions of zinc, genotype and sowing date at the two locations over the 2 years. For the β-ODAP content, Table 1 shows that the main effects of the factors genotype, sowing date and zinc and their interactions were significant ($P < 0.001$). Interactions of these factors were significant except for the three-factor location × sowing date × genotype interaction. Among the interactions involving years within locations, only the year × genotype interaction was significant. On the seed yield as well, the various main effects and interactions of the experimental factors and locations listed above were significant. In addition, the three-factor interactions between sowing date, zinc and year within locations were also significant ($P < 0.05$). Further, the partition of variation due to zinc levels into linear and quadratic components showed high accountability by the linear components in all the significant interactions. Quadratic components were significant in a few cases while deviations from the quadratic model were generally insignificant. Since the variation due to zinc can be largely described by the linear component, therefore, this provided a basis for modelling the response to zinc as a linear function for the β-ODAP synthesis and yield.

Table 1. Significance in terms of P-value for main effects and interactions of applied zinc, sowing dates, varieties and interactions on β-ODAP content and yield

		β-ODAP (%)			Yield		
Source	DF	Zinc, overall	Zinc, linear	Zinc, quadratic	Zinc, overall	Zinc, linear	Zinc, quadratic
Location	1	NV	–	–	NV	–	–
Years (Yr) within (wtn) location	3	NV	–	–	NV	–	–
Replications within Years within Location (Loc)	8	–	–	–	–	–	–
Sowing Date (SD)	1	<0.001	–	–	<0.001	–	–
Loc × SD	1	0.128	–	–	0.663	–	–
SD × Yr within location	2	0.664	–	–	0.012	–	–
Residual	8	–	–	–	–	–	–
		–	–	–	–	–	–
Genotype (G)	1	<0.001	–	–	<0.001	–	–
Zinc (Z)	4	<0.001	<0.001	0.779	<0.001	<0.001	0.009
G × Z	4	<0.001	<0.001	0.024	<0.001	<0.001	0.011
SD × G	1	<0.001	<0.001	–	<0.001	–	–
SD × Z	4	<0.001	0.002	0.007	<0.001	<0.001	0.069
SD × G × Z	4	<0.001	<0.001	0.447	<0.001	<0.001	0.016
Loc × G	1	<0.001	–	–	<0.001	–	–
Loc × Z	4	<0.001	<0.001	0.779	<0.001	<0.001	<0.001
Loc × G × Z	4	<0.001	<0.001	<0.001	0.001	0.839	<0.001
Loc × SD × G	1	0.644	–	–	0.172	–	–
Loc × SD × Z	4	<0.001	<0.001	0.057	0.020	0.007	0.060
Loc × SD × G × Z	4	<0.001	0.079	(<0.001)[†]	0.004	0.007	(0.043)[†]
Yr × G wtn Loc	2	<0.001	–	–	<0.001	–	–
Yr × Z wtn Loc	8	0.14	0.002	1.00	0.002	<0.001	0.147
Yr × G × Z wtn Loc	8	0.872	0.164	(1)[†]	0.327	0.327	(0.379)[†]
Yr × SD × V wtn Loc	2	0.673	–	–	0.292	–	NS
Yr × SD × Z wtn Loc	8	1	0.97	(1)[†]	0.816	0.998	(0.618)[†]
Yr × SD × G × Z wtn Loc	8	1	(1)[†]	–	0.653	–	–

NV, not valid (due to no randomisation).
β-ODAP unweighted, yield based on weighted ANOVA.
[†] Includes deviation terms.

β-ODAP (%) for the improved variety was much lower than that for the local variety at any applied dose of zinc (Table 2). With application of 20 kg ha^{-1} zinc on the improved variety, β-ODAP (%) reduced from 0.15% to 0.088% at Debre Zeit and 0.14% to 0.08% at Sheno. The reduction for the local variety was from 0.58% to 0.33% at Debre Zeit and 0.43% to 0.27% at Sheno. Early sowing resulted in lower β-ODAP irrespective of genotype and location. β-ODAP content was found to reduce with zinc level for each combination of genotype, time of planting and location. Similar trend in decline of β-ODAP with zinc level was also found while working with a set of nine grasspea genotypes.[15]

Yield of the improved variety was much higher than that of the local variety at any applied dose of zinc (Table 3). With application of 20 kg ha^{-1} zinc on the improved variety, yield increased from 841 to 2260 kg ha^{-1} at Debre Zeit and from 715 to 1835 kg ha^{-1} Sheno. The similar increase for the local variety was from 833 to 1533 kg ha^{-1} at Debre Zeit and 582 to 968 kg ha^{-1} at Sheno. Early planting is advantageous for grain yield in each combination of genotype, zinc level and location. It was statistically significant ($P < 0.05$) for improved genotypes at all the combinations of zinc levels and location except at Debre Zeit when no zinc was applied, while for the local landrace increase in yield due to early planting was not statistically significant except when 10 kg ha^{-1} zinc was applied at Debre Zeit and 15 kg ha^{-1} at Sheno. The improved genotype responds significantly to the time of planting in reducing the β-ODAP content and increasing the yield.

Following the strong significance of linear components (Table 4), the regression line in zinc doses was fitted for each combination of location, sowing date and variety, and for the varieties overall and the relationships are shown in Fig. 1. The linear fit for these combinations showed percentage variance accounted for in the range 69–99% for β-ODAP and 89–98% for the yield and the slopes were statistically significant ($P < 0.05$). The effect of zinc in reducing the β-ODAP is shown by the negative slope in each case. The reliability of extrapolation of doses above 20 kg ha^{-1} can always be questioned. Further experimentation with higher doses is needed. However, since 20 kg ha^{-1} zinc is affordable to poor and marginal farmers and since the relationships of the β-ODAP content and yields are linear, the maximum affordable level is recommended.

DISCUSSION

This study showed variation in the β-ODAP due to variety, location and sowing date. Across the two locations, the mean content varied in the range 0.14 –0.15% for the improved variety while 0.43–0.58% for the local variety. These values are in the range 0.20 to 0.45% reported for Debre Zeit, 2006/2007.[18,24] The improved variety in this study showed a slightly lower level of β-ODAP when no zinc was applied in comparison with other studies.

In the field trial conducted at Tel Hadya, northern Syria, to examine the effect and interactions of zinc, phosphorus and

Table 2. Mean β-ODAP % response of zinc application on variety and sowing date at two locations in Ethiopia (combined over 2 years, 2002 and 2003)

			Zinc (kg ha^{-1})				
Location	Genotype	SD	0	5	10	15	20
Debre-Zeit	G1 local	D1	0.522	0.480	0.431	0.361	0.298
		D2	0.644	0.585	0.466	0.431	0.371
		Mean	0.583	0.532	0.448	0.396	0.334
	G2 improved	D1	0.137	0.109	0.116	0.084	0.077
		D2	0.168	0.158	0.137	0.130	0.100
		Mean	0.152	0.133	0.126	0.107	0.088
	Mean		0.368	0.333	0.287	0.251	0.211
Sheno	G1	D1	0.401	0.344	0.319	0.287	0.229
		D2	0.456	0.434	0.398	0.340	0.297
		Mean	0.428	0.389	0.358	0.314	0.267
	G2	D1	0.125	0.111	0.090	0.079	0.061
		D2	0.158	0.122	0.104	0.104	0.100
		Mean	0.142	0.116	0.097	0.091	0.081
	Mean		0.285	0.253	0.228	0.202	0.172
Standard errors of means in the various factor combinations							
			Z × SD × G	Z × SD	Z × G	SD × G	G
Debre-Zeit			0.0054 (0.0052)	0.0040 (0.0037)	0.0037	0.0028 (0.0023)	0.0017
Sheno			0.0056 (0.0041)	0.0048 (0.0029)	0.0029	0.0042 (0.0018)	0.0013

Z, zinc, SD = sowing date, G = genotype.
Genotypes: G1 = Debre-Zeit local variety; G2 = ILAT-LS-B10-520.
Sowing dates D1 = Sowing date 1 (29 August 2001 and 2002), D2 = Sowing date 2 (12 September 2001 and 2002), both locations.

Table 3. Mean yield response (kg ha^{-1}) of zinc application on variety and sowing date at two locations in Ethiopia (combined over the 2 years)

			Zinc (kg ha^{-1})				
Location	Genotype	SD	0	5	10	15	20
Debre-Zeit	G1	D1	896	1085	1306|	1328	1477
		D2	770	951	1090	1221	1588
		Mean	833	1018	1198	1274	1532
	G2	D1	925	1352	1585	2218	2538
		D2	758	968	1173	1497	1983
		Mean	841	1160	1379	1858	2260
	Mean		837	1089	1289	1566	1896
Sheno	G1	D1	615	670	833	1006	1143
		D2	548	614	744	776	793
		Mean	582	642	789	891	968
	G2	D1	787	1283	1782	2037	2083
		D2	644	982	1203	1400	1587
		Mean	715	1133	1493	1718	1835
	Mean		648	887	1141	1305	1401
Standard errors of means in the various factor combinations							
			Z × SD × G	*Z × SD*	*Z × G*	*SD × G*	*G*
Debre-Zeit			59 (61)	40(43)	43	21(28)	19
Sheno			44 (42)	32(30)	30	22(19)	13

Z: Zinc, SD = Sowing date, G = Genotype
Genotypes: G1 = Debre-Zeit local variety; G2 = ILAT-LS-B10-520;
Sowing dates D1 = Sowing date 1 (29 August 2001 and 2002), D2 = Sowing date 2 (12 September 2001 and 2002), both locations

grasspea genotypes on β-ODAP and yield,[15] estimates were substantially higher in comparison with those found in this study. Based on the nine genotypes, β-ODAP ranged from 0.25% to 0.57% in the absence of zinc and from 0.21% to 0.49% in the presence of 20 kg ha^{-1} Zn,[15] while in this study (Table 2) it ranged from 0.14% to 0.58% and 0.08% to 0.33%, respectively, in the absence and presence of the above levels of zinc application. While application of zinc showed a significant reduction in β-ODAP in Ethiopian conditions, the reduction was not that substantial at Tel Hadya.

Table 4. Estimated linear equations in zinc for β-ODAP % and yield for variety, sowing date and combinations with location

			β-ODAP %			Yield (kg ha^{-1})		
Location	Sowing date	Genotype	Intercept	Slope	Adj. R^2 %	Intercept	Slope	Adj. R^2 %
Debre Zeit	29 August	Debre Zeit Local	0.5313	−0.01134	98.7	937.3	28.09	92.9
		ILAT-LS-520	0.133	−0.00287	84.1	904.9	81.85	97.8
	12 September	Debre Zeit Local	0.6391	−0.014	96.0	742.7	38.13	93.7
		ILAT-LS-520	0.1712	−0.00329	94.7	679.7	59.61	94.9
Sheno	29 August	Debre Zeit Local	0.3963	−0.00803	97.3	575.2	27.82	97.1
		ILAT-LS-520	0.1254	−0.00323	99.0	925	66.93	89.2
	12 September	Debre Zeit Local	0.4666	−0.00817	96.8	565	13.01	87.9
		ILAT-LS-520	0.1441	−0.00265	68.8	702.2	46.09	97.8
Over both locations and sowing dates		Debre Zeit Local	0.5083	−0.01038	99.8	705.1	26.76	99.1
		ILAT-LS-520	0.1434	−0.00301	98.2	802.9	63.62	99.6

Adj. R^2 % = % variance accounted for (residual degrees of freedom = 3).

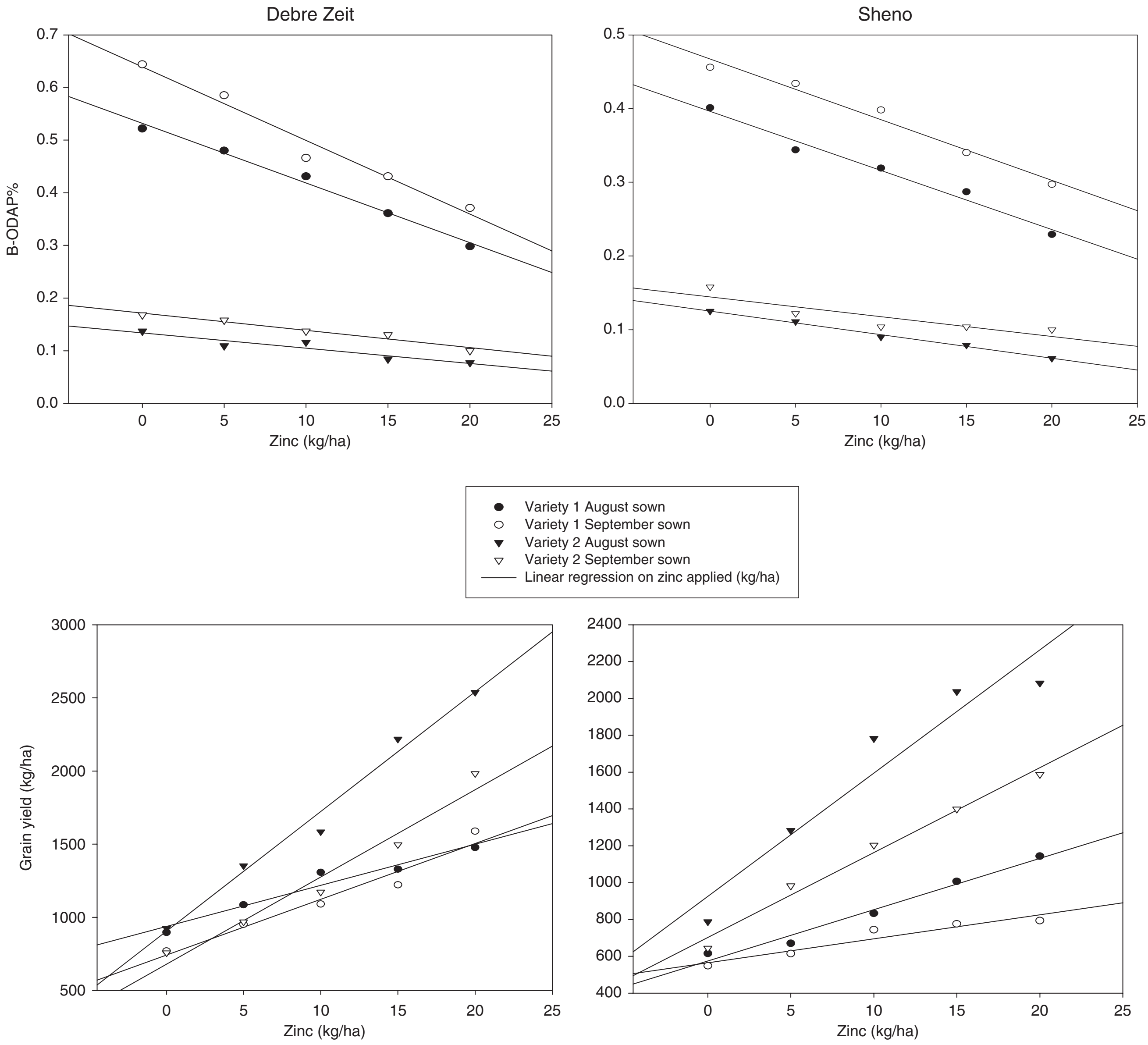

Figure 1. β-ODAP (%) and grain yield (kg ha^{-1}) response to application of zinc for two varieties sown in August and September 2001/2002 and 2002/2003 at two locations – Debre Zeit and Sheno – in Ethiopia.

The study conducted at Debre Zeit in 2001/02–2002/03 showed difference in the estimates for the local and the improved line, over the 2 years: 0.28–0.34% for the local and 0.16–0.30% for the improved variety, in which case the two varieties were sown over two different sets of dates, therefore, the variety comparison is confounded with the set of dates.[16] As a further exploration to estimating the effect of zinc on the toxicity and yield of grasspea,[15] this study modelled their relationships with the zinc which guided on the amount of zinc that can be applied to contain the toxicity and increase the yield for specified target levels.

CONCLUSION

With the data obtained from the experimental conducted at two representative locations for Ethiopian conditions, this study has summarised the effects and interactions of grasspea variety, sowing date and zinc on the anti-nutrition content of β-ODAP and grain yield. It also modelled β-ODAP and grain relationships with zinc amount. The above factors showed significant main effects and two factor interactions. An early sowing favoured the response on β-ODAP and yield. The linear response relationship with zinc was found to increase for yield and decrease for β-ODAP, which can help determine the zinc dose for a target levels of the β-ODAP and or the yield.

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