

## Implications of Zinc Fertilization for Ameliorating Toxicity (Neurotoxin) in Grasspea (*Lathyrus sativus*)

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### ABSTRACT

Grasspea (*Lathyrus sativus*) is a protein-rich forage legume that contains a neurotoxin (beta-ODAP) that causes severe malformation of the body's extremities when eaten in large quantities. Various low-toxin lines of grasspea have been developed to reduce the toxin's concentration in the grain, i.e. to below the 0.2% critical value, but there are indications that zinc (Zn) can also reduce the toxin. Thus, we assessed the effect of added Zn, using a low Zn (0.4 mg kg<sup>-1</sup> DTPA Zn) soil, on B-ODAP in several grasspea lines in two greenhouse experiments and three field trials on the same soil type from northern Syria. In the greenhouse, Zn consistently reduced the toxin in three lines in the first experiment but not in the second one with some high-toxin lines, while added P had no effect on Zn. In the field trial for three seasons (260, 429, and 405 mm rainfall) with nine grasspea lines, applied Zn (0, 5, 10, 20 kg ha<sup>-1</sup>) tended to reduce B-ODAP in the grain by 10 to 40%. There was no relationship between Zn concentrations in the grasspea grain or straw and the reduction in B-ODAP. The mechanism by which the B-ODAP concentration may be reduced by added Zn is not known. The effect of Zn is only a partial solution at best to the problem of neurolathyrism; breeding grasspea lines that have no harmful levels of the toxin is required.

**Keywords:** Forage/food legumes, Grasspea, Neurolathyrism, Zinc fertilizer.

### INTRODUCTION

While agricultural science has contributed technologies that helped increase food output at the global level (Stewart *et al.*, 2005), hunger and malnutrition are still endemic in most of Africa and parts of Asia (Borlaug, 2003) where a range of biophysical factors such as drought, low soil fertility, and water-logging pose severe constraints to production, in addition to many socio-economic factors (poverty, limited health care, etc). While some indigenous crops are adapted to harsh environmental conditions, the problem of alleviating hunger is often compounded by soil factors that impinge on human health

(Oliver, 1997). Indeed, most overviews of plant nutrition deal with direct effects of nutrients rather than indirect effects (Loneragan, 1977). A typical example of innate soil/plant factors that serve as anti-nutritional components is exemplified by grasspea (*Lathyrus sativus*) which is a crop of "last resort" in times of famine in countries such as Ethiopia.

*Lathyrus* belongs to the *Vicia* tribe, which includes common Mediterranean legumes such as pea (*Pisum sativa*), faba bean (*Vicia faba*), and lentil (*Lens culinaris*). The genus contains 160 species and 45 subspecies embracing food, forage, and environmental crops. Grasspea cultivation has been widely practised as a food and feed crop for millennia in Africa, especially

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Ethiopia, and in South Asia, e.g. in India, China, Pakistan, Nepal, and Bangladesh (Bell, 1989). The crop is known by various local/regional names, such as *Khessari* in India and Bangladesh, *Guaya* in Ethiopia, and *san li dow* in China. As it is a hardy, drought-tolerant subsistence crop, it serves as a human food source in times of famine. Grasspea survives and yields relatively well on low-fertility soils and as a legume adds nitrogen (N) to the soil through N symbiotic fixation. While grasspea seeds are nutritious, with about 30% protein, it is generally the cheapest food legume for low income families, being a major component of their diet (Alitor *et al.*, 1994). Since, grasspea contains a high concentration of free L-homoargmine, a precursor for lysine, it is a valuable animal feed (Quereshi *et al.*, 1977).

Notwithstanding its agronomic advantages, grasspea has a major nutritional drawback since it contains a neurotoxic non-protein amino acid that causes irreversible spastic paraparesis (paralysis) of the legs if consumed in large quantities for extended periods. Outbreaks of this motorneurone disease have occurred at various periods of food scarcity in Bangladesh, China and Ethiopia (Haque *et al.*, 1991; Lambien *et al.*, 1993; 1994; Liu and He, 1990; Haimanat *et al.*, 1990; Hanbury *et al.*, 2000). The causal agent of this food-induced affliction was identified as 3-(N-oxalyl)-L-2, 3-diamino propionic acid (B-ODAP) or its synonym B-N oxalyl-L-alanine (BOAA) and its biochemical pathway has been elucidated (Lambien *et al.*, 1993, 1994). While no role of B-ODAP in the plant has been established, its concentration is influenced by both the specific *L. sativus* lines or cultivars and the environment; water stress can double toxin levels (Hanbury *et al.*, 1999). Although Zn deficiency has been associated with neuro-lathyrism (Lambien *et al.*, 1994), the exact mechanism is unclear.

Despite the geographical extent of neuro-lathyrism, little concerted effort was made

to alleviate the problem until recently, when genotype-environment interactions of *lathyrus* were examined (Hanbury *et al.*, 1999; Abd El-Moneim and Cocks, 1993), with a focus on breeding to reduce B-ODAP levels in grasspea (Campbell *et al.*, 1994) to relatively safe levels (below 0.2%). The International Center for Agricultural Research in the Dry Areas (ICARDA) initiated a breeding program to address *lathyrus* toxicity, especially as grasspea is adapted to its mandate region (Abd El-Moneim and Cocks, 1993) and as it deals with Ethiopia where lathyrism is common.

The initial connection between Zn and neuro-lathyrism stemmed from the incidence of the disease in soils low or depleted in plant available Zn, such as in Bangladesh (Mannan and Rahim, 1988). In addition, evidence showed Zn deficiency to be implicated in motor-neurone disease (De Belleruche and Clifford, 1987) and that B-ODAP may be a carrier for Zn (Lambien *et al.*, 1994); even though no physiological role of B-ODAP has been identified. Such medical evidence led Lambien *et al.* (1994) to hypothesize that Zn deficiency can make an individual who consumes grasspea in considerable quantities more susceptible to the toxic action of B-ODAP or can lower the threshold for B-ODAP toxicity. As Zn is connected in some obscure way with the disease and frequently occurs in low-Zn soils (Hanbury *et al.*, 2000; Mannan and Rakim, 1988), we established a series of experiments in the greenhouse and the field to assess the possible effect of added Zn on B-ODAP concentrations of established grasspea lines (cultivars and selections) from ICARDA's improvement program.

## MATERIALS AND METHODS

The research on B-ODAP of grasspea involved two preliminary experiments in the greenhouse and three field experiments.

### Greenhouse Experiments (2): Grasspea Lines vs. Added Zinc and Phosphorus

The soil used was the dominant soil type at ICARDA's main station at Tel Hadya, Aleppo, which is fine clay, thermic, Calcixerollic Xerochrept (Ryan *et al.*, 1997). Following air-drying, the soil was put through a 6-mm sieve and 3.5 kg lots placed in plastic pots. To each pot was added Zn at 0, 10 and 50 mg kg<sup>-1</sup> mixed with the soil as zinc sulphate (ZnSO<sub>4</sub>·7H<sub>2</sub>O, 22% Zn). Other nutrients, nitrogen (N) and phosphorus (P), were added to the soil in adequate amounts (20 mg kg<sup>-1</sup>). The soil was low in available Zn at 0.4 mg kg<sup>-1</sup> DTPA-Zn (Lindsay and Norvell, 1978) (All other nutrients were adequate according to test value norms and therefore were not added). Three grasspea lines were planted (5 plants per pot, with three replicates), which represented the range in toxicity LS 512, LS 560, and LS 562. The design was a split-strip with grasspea lines being the main plots and Zn the sub-plots. The pots were then watered to field capacity, and subsequently as required, and the crop was grown for 126 days (20/1/1998 to 16/5/1998), following which the plants were harvested, dried, weighed, and analyzed for B-ODAP (Briggs *et al.*, 1983).

A second and similarly arranged greenhouse experiment involved two high-toxin lines (Ethiopia 1 and 2) and a low-toxin line from ICARDA (LS 288). The Zn treatment was expanded to 0, 5, 10, 25, and 50 mg kg<sup>-1</sup>. While N was added as a basal dressing (20 mg kg<sup>-1</sup>), P was a variable with application of 10 and 50 mg kg<sup>-1</sup> as monocalcium phosphate to the soil which contained 6 mg kg<sup>-1</sup> Olsen-P. The rationale for including P is based on the possible negative influence of P on Zn availability (Olsen, 1972). The experimental design was a split-split plot with lines as the main plots and Zn and P the sub-plots. With adequate and periodic watering, the plants were grown for 125

days (24/2/1999 to 29/6/1999) and then harvested, dried and analyzed.

### Field Trials (3): Grasspea Lines vs Zn

All three experiments (1999-20000, 2000-2001, and 2001-2002) were conducted on the same soil type at Tel Hadya. The Zn applications of 0, 5, 10, and 20 mg kg<sup>-1</sup> were hand-broadcasted at pre-planting as zinc sulphate and mixed into the soil during subsequent cultivation. The number of grasspea lines were expanded to nine (Accessions 190, 288, and 390 and Selections 716, 722, 769, 502, 670, and 683), including low medium and high original B-ODAP. In the three growing seasons under rainfed conditions normal to the Mediterranean region, the mean annual rainfall was 260 mm in 1999-20000, 429 mm in 2000-2001, and 405 mm in 2001-2002.

As P had no effect on Zn in the greenhouse, it was not variable in the field trial and was applied as a blanket dressing of 30 kg P ha<sup>-1</sup> as triple superphosphate. The agronomic practices were similar in each year. Sowing the seed by drill followed standard soil cultivation in the fall (November-December), with seed covering by harrow. Each trial was in a different part of a homogenous field, and each year the legume crop followed a wheat crop in the previous year (In each site, the level of DTPA-Zn was above 1.0 mg kg<sup>-1</sup> in contrast to the greenhouse soil which was 0.4 mg kg<sup>-1</sup>). During the season, weeds were controlled chemically. Prior to harvest in May during the hay stage, fresh samples of the standing crop were taken for B-ODAP analysis (Briggs *et al.*, 1983). Following harvest, grain and straw yields were recorded along with Zn concentrations in both grain and straw.

The design of the three field trials was a split-plot with three replications. The sowing was done mechanically by an Ojyord planter using a seed rate of 250 seeds m<sup>-2</sup> per plot (1.2×30 m) and a row spacing of 30 cm.



The planting dates were November 30, 1999; December 12, 2000, and December 22, 2001, while the respective harvest dates were May 25 in 2000, May 20 in 2001, and May 18 in 2002.

## RESULTS

### Greenhouse

The first greenhouse study indicated an effect of applied Zn on the parameters measured, but with differences between the *lathyrus* lines (Table 1). For seed weight there was a significant increase to Zn in two lines, LS 512 and LS 562, but no consistent response for LS 560. Added Zn significantly decreased B-ODAP in the seeds of the three lines; however, these decreases were poorly related to increased yields.

The second greenhouse study examined the effect of Zn on three different grasspea lines, all of which had B-ODAP levels much higher (Table 2) than in the first study. Although P was a factor in the study, there was no consistent effect of P on either yield components (not presented), nor on B-ODAP in either grain or straw. Despite the absence of a Zn effect on yield, there were some apparent effects of added Zn, but the overall effect of Zn was not significant. In

all cases, increasing the amount of Zn decreased the concentration of B-ODAP in the grain of the three grasspea lines; reductions were between 10-20% of the control values without added Zn. In contrast to the grain, there was no obvious effect of Zn on B-ODAP in the straw of any line.

### Field Trials

In the first season (Table 3), the grasspea lines varied widely in yield, with lines that were low in B-ODAP showing the lowest yield (190, 288, and 390) compared to lines with B-ODAP levels over 0.40%. Yields of two high-B-ODAP lines (796, 502) had almost double the yields of the low B-ODAP lines. The influence of Zn on grain yield varied with the grasspea line. In some cases, added Zn increased yields (716) while for most lines the effect was absent or inconsistent. Added Zn gave a small but consistent decrease in B-ODAP (190, 288, and 390) but had no consistent effect for the relatively high B-ODAP lines.

In the second season in which the same grasspea lines were grown (Table 4), there was no consistent effect of added Zn on grain yield. However, levels of B-ODAP in the grasspea grain were generally lower than the previous season but with the same

**Table 1.** Zinc application in relation to grasspea parameters.

| <u>Zinc</u>          | <u>Grasspea lines</u>                    |               |               | <u>Mean</u> | <u>SE</u> |
|----------------------|------------------------------------------|---------------|---------------|-------------|-----------|
|                      | <u>LS 512</u>                            | <u>LS 560</u> | <u>LS 562</u> |             |           |
| -mg kg <sup>-1</sup> | -----100-seed weight (g)-----            |               |               |             |           |
| 0                    | 7.4                                      | 13.5          | 11.2          | 10.7        | 3.0       |
| 5                    | 9.7                                      | 11.2          | 12.3          | 11.4        | 1.5       |
| 50                   | 10.4                                     | 13.5          | 12.2          | 12.0        | 1.6       |
|                      | -----Dry matter yield/25 plants (g)----- |               |               |             |           |
| 0                    | 29.9                                     | 52.3          | 48.2          | 43.5        | 11.9      |
| 5                    | 49.4                                     | 57.0          | 53.9          | 51.5        | 2.3       |
| 50                   | 63.9                                     | 70.9          | 56.8          | 63.9        | 7.0       |
|                      | -----B-ODAP %-----                       |               |               |             |           |
| 0                    | 0.25                                     | 0.33          | 0.28          | 0.29        | 0.04      |
| 5                    | 0.19                                     | 0.20          | 0.21          | 0.20        | 0.08      |
| 50                   | 0.15                                     | 0.16          | 0.17          | 0.16        | 0.02      |

Note: Original data not available for statistical analyses due to unforeseen circumstances.

**Table 2.** Phosphorus and zinc fertilization on toxin (B-ODAP) concentration in grasspea lines.

| Phosphorus                     | Zinc | Ethiopian 1 |       | Ethiopian 2 |       | LS 288 |       |
|--------------------------------|------|-------------|-------|-------------|-------|--------|-------|
| -----mg kg <sup>-1</sup> ----- |      | Grain       | Straw | Grain       | Straw | Grain  | Straw |
|                                |      | -----%----- |       |             |       |        |       |
| 0                              | 0    | 0.79        | 0.75  | 0.77        | 0.97  | 0.33   | 0.66  |
| 0                              | 5    | 0.79        | 0.80  | 0.69        | 0.96  | 0.29   | 0.82  |
| 0                              | 10   | 0.76        | 0.68  | 0.66        | 0.98  | 0.29   | 0.73  |
| 0                              | 25   | 0.67        | 0.78  | 0.66        | 1.19  | 0.27   | 0.86  |
| 0                              | 50   | 0.73        | 0.80  | 0.65        | 0.94  | 0.25   | 0.01  |
| Mean                           |      | 0.73        | 0.75  | 0.68        | 1.01  | 0.29   | 0.85  |
| SE                             |      | 0.03        | 0.02  | 0.02        | 0.04  | 0.01   | 0.08  |
| 10                             | 0    | 0.76        | 0.78  | 0.88        | 0.88  | 0.43   | 0.67  |
| 10                             | 5    | 0.70        | 0.77  | 0.77        | 0.90  | 0.30   | 0.72  |
| 10                             | 10   | 0.68        | 0.75  | 0.69        | 0.89  | 0.29   | 0.41  |
| 10                             | 25   | 0.66        | 0.72  | 0.63        | 0.98  | 0.28   | 0.90  |
| 10                             | 50   | 0.61        | 0.76  | 0.62        | 0.88  | 0.25   | 0.91  |
| Mean                           |      | 0.68        | 0.76  | 0.72        | 0.88  | 0.31   | 0.80  |
| SE                             |      | 0.02        | 0.01  | 0.04        | 0.11  | 0.03   | 0.01  |
| 50                             | 0    | 0.80        | 0.96  | 0.81        | 1.04  | 0.48   | 0.84  |
| 50                             | 5    | 0.77        | 0.91  | 0.68        | 0.82  | 0.23   | 1.09  |
| 50                             | 10   | 0.60        | 0.80  | 0.61        | 0.93  | 0.23   | 0.79  |
| 50                             | 25   | 0.66        | 0.84  | 0.64        | 1.11  | 0.20   | 0.84  |
| 50                             | 50   | 0.67        | 0.89  | 0.75        | 1.17  | 0.38   | 0.86  |
| Mean                           |      | 0.70        | 0.88  | 0.70        | 1.02  | 0.30   | 0.88  |
| SE                             |      | 0.01        | 0.02  | 0.03        | 0.06  | 0.05   | 0.05  |

LSD (1%) for Varieties= 0.12% (Grain); 0.09% (Straw); LSD (5%) for Zn×P= 0.01, LSD (5%) for Zn, P= N. S. (Non significant).

ranking between lines. Again, as in the first season, added Zn consistently reduced B-ODAP but this time most lines had reduced levels of the neurotoxin; in most cases, the reduction was 10-40% of the original value without Zn.

Analyses of Zn in the fresh material DM (hay), straw and grain of the nine grasspea lines from the second season indicated

significant differences between lines in terms tissue of Zn concentrations in grasspea hay and straw with added Zn (Table 5), but no significant effect for grain. There was little effect of added Zn on the Zn concentration in either yield component.

In the third and final evaluation of the nine grasspea lines in the field, all lines showed a consistent but non-significant decrease in the

**Table 3.** Influence of Zn on toxin (B-ODAP) concentration and grain yield of grasspea lines (1999-2000).

| Grasspea lines | Zn, kg ha <sup>-1</sup> |      |      |      |      |       | Zn, kg ha <sup>-1</sup>                     |      |      |      |      |        |
|----------------|-------------------------|------|------|------|------|-------|---------------------------------------------|------|------|------|------|--------|
|                | 0                       | 5    | 10   | 20   | Mean | SE±   | 0                                           | 5    | 10   | 20   | Mean | SE±    |
|                | -----B-ODAP %-----      |      |      |      |      |       | -----Grain yield, kg ha <sup>-1</sup> ----- |      |      |      |      |        |
| 190            | 0.25                    | 0.23 | 0.22 | 0.21 | 0.22 | 0.008 | 747                                         | 661  | 743  | 653  | 701  | 25.47  |
| 288            | 0.37                    | 0.37 | 0.33 | 0.30 | 0.34 | 0.016 | 679                                         | 940  | 723  | 801  | 786  | 57.27  |
| 390            | 0.39                    | 0.36 | 0.35 | 0.33 | 0.35 | 0.011 | 908                                         | 918  | 810  | 852  | 872  | 25.26  |
| 716            | 0.47                    | 0.47 | 0.44 | 0.40 | 0.44 | 0.016 | 1039                                        | 1419 | 1379 | 1501 | 1334 | 101.72 |
| 722            | 0.51                    | 0.50 | 0.42 | 0.43 | 0.46 | 0.024 | 1162                                        | 1034 | 1090 | 1247 | 1133 | 46.09  |
| 796            | 0.50                    | 0.51 | 0.54 | 0.44 | 0.50 | 0.022 | 1425                                        | 1267 | 1083 | 1408 | 1327 | 79.26  |
| 502            | 0.57                    | 0.53 | 0.56 | 0.49 | 0.54 | 0.018 | 1347                                        | 1355 | 1280 | 1298 | 1320 | 18.34  |
| 670            | 0.54                    | 0.53 | 0.50 | 0.48 | 0.51 | 0.013 | 1129                                        | 900  | 913  | 997  | 985  | 52.67  |
| 683            | 0.54                    | 0.53 | 0.47 | 0.46 | 0.49 | 0.021 | 1031                                        | 1104 | 1001 | 1142 | 1070 | 32.43  |

ODAP%: LSD (1%) for Lines= 0.06; LSD (5%) for Zn= 0.04; Lines×Zn= N. S. (Non significant).

Grain yield: LSD (1%) for lines=183; Zn = N.S; lines×Zn =N. S. (Non significant).

**Table 4.** Dry matter yield and B-ODAP concentrations in grain of grasspea lines (2000-2001).

| Zinc                | LS 190                                      | LS 288 | LS 390 | LS 716 | LS 722 | LS 736 | LS 502 | LS 670 | LS 683 |
|---------------------|---------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| kg ha <sup>-1</sup> |                                             |        |        |        |        |        |        |        |        |
|                     | -----Grain yield, kg ha <sup>-1</sup> ----- |        |        |        |        |        |        |        |        |
| 0                   | 279                                         | 603    | 572    | 1176   | 1148   | 1649   | 1322   | 1082   | 1376   |
| 5                   | 462                                         | 678    | 775    | 1371   | 1257   | 1507   | 1102   | 1021   | 1247   |
| 10                  | 230                                         | 618    | 520    | 1451   | 1282   | 1451   | 1045   | 915    | 1121   |
| 20                  | 378                                         | 640    | 661    | 1321   | 1365   | 1320   | 1333   | 1071   | 1306   |
| Mean                | 337                                         | 634    | 632    | 1329   | 1263   | 1481   | 1208   | 1022   | 1262   |
| SE±                 | 38                                          | 62     | 52     | 41     | 22     | 46     | 71     | 48     | 36     |
|                     | -----B-ODAP %-----                          |        |        |        |        |        |        |        |        |
| 0                   | 0.19                                        | 0.29   | 0.29   | 0.27   | 0.36   | 0.32   | 0.42   | 0.47   | 0.42   |
| 5                   | 0.18                                        | 0.27   | 0.26   | 0.27   | 0.26   | 0.29   | 0.38   | 0.32   | 0.33   |
| 10                  | 0.16                                        | 0.26   | 0.26   | 0.26   | 0.30   | 0.26   | 0.32   | 0.26   | 0.30   |
| 20                  | 0.14                                        | 0.25   | 0.24   | 0.24   | 0.27   | 0.26   | 0.31   | 0.24   | 0.27   |
| Mean                | 0.17                                        | 0.27   | 0.26   | 0.26   | 0.30   | 0.28   | 0.35   | 0.32   | 0.33   |
| SE±                 | 0.011                                       | 0.008  | 0.011  | 0.007  | 0.024  | 0.013  | 0.026  | 0.052  | 0.032  |

Grain yield: LSD (1%) for lines= 230; Zn= N. S. (Non significant), Lines×Zn= N. S. (Non significant)

B-ODAP%: LSD (1%) for lines= 0.05; Zn= N. S. (Non significant), Lines×Zn= N. S. (Non significant)

**Table 5.** Zinc concentrations in hay, straw and grain of grasspea lines in relation to added zinc (2000-2001).

| Zinc                | L.S 190                                   | L.S 288 | L.S 390 | L.S 716 | L.S 722 | L.S 736 | L.S 502 | L.S 670 | L.S 683 |
|---------------------|-------------------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| kg ha <sup>-1</sup> |                                           |         |         |         |         |         |         |         |         |
|                     | -----Hay, Zn, mg kg <sup>-1</sup> -----   |         |         |         |         |         |         |         |         |
| 0                   | 17.8                                      | 17.3    | 17.3    | 19.6    | 20.5    | 22.5    | 19.0    | 17.0    | 16.5    |
| 5                   | 16.0                                      | 18.6    | 20.0    | 17.8    | 18.1    | 19.6    | 19.8    | 20.5    | 16.6    |
| 10                  | 20.3                                      | 24.8    | 21.8    | 20.5    | 20.1    | 19.3    | 16.6    | 21.9    | 14.1    |
| 20                  | 17.5                                      | 22.5    | 19.8    | 18.3    | 19.1    | 21.3    | 18.5    | 17.6    | 15.1    |
| Mean                | 17.9                                      | 20.8    | 19.7    | 19.8    | 19.5    | 20.7    | 18.5    | 19.0    | 15.6    |
| SE±                 | 0.89                                      | 1.71    | 0.92    | 0.61    | 0.52    | 0.75    | 0.66    | 1.00    | 0.59    |
|                     | -----Straw, Zn, mg kg <sup>-1</sup> ----- |         |         |         |         |         |         |         |         |
| 0                   | 28.5                                      | 33.5    | 32.5    | 30.8    | 32.0    | 32.0    | 33.0    | 32.5    | 31.3    |
| 5                   | 30.1                                      | 30.8    | 32.6    | 32.0    | 30.3    | 29.1    | 30.8    | 34.0    | 28.8    |
| 10                  | 29.3                                      | 31.8    | 30.1    | 30.8    | 29.3    | 29.8    | 33.0    | 31.0    | 30.3    |
| 20                  | 29.3                                      | 31.5    | 31.7    | 32.5    | 29.6    | 31.8    | 31.5    | 30.8    | 30.8    |
| Mean                | 29.3                                      | 31.9    | 31.6    | 31.5    | 30.3    | 30.7    | 32.0    | 32.0    | 30.3    |
| SE±                 | 0.34                                      | 0.56    | 0.59    | 0.42    | 0.59    | 0.72    | 0.54    | 0.74    | 0.54    |
|                     | -----Grain, Zn, mg kg <sup>-1</sup> ----- |         |         |         |         |         |         |         |         |
| 0                   | 10.8                                      | 12.50   | 15.8    | 12.4    | 12.3    | 13.0    | 16.0    | 12.8    | 11.4    |
| 5                   | 17.83                                     | 17.5    | 13.0    | 11.3    | 14.6    | 14.1    | 17.3    | 20.5    | 13.0    |
| 10                  | 14.17                                     | 24.5    | 23.3    | 15.1    | 12.0    | 14.5    | 14.1    | 20.5    | 13.3    |
| 20                  | 23.33                                     | 17.1    | 17.6    | 12.8    | 12.1    | 15.1    | 19.5    | 19.8    | 18.1    |
| Mean                | 16.54                                     | 17.9    | 17.6    | 12.9    | 12.7    | 14.2    | 16.7    | 18.4    | 13.9    |
| SE±                 | 2.67                                      | 2.47    | 2.18    | 0.80    | 0.62    | 0.45    | 1.12    | 1.86    | 1.45    |

Hay: LSD (5%) for lines = 2.70; Zn= N. S. (Non significant), Lines×Zn = N. S. (Non significant).

Straw: LSD (5%) for lines = 1.74, Zn = N.S.; Lines×Zn = N. S. (Non significant).

Grain: No significant effects.

grain B-ODAP concentration with added Zn (Table 6). The maximum reduction at the highest Zn application rate ranged from about 15 to 30%. The relationship between added Zn and B-ODAP concentration in hay and straw showed little consistency.

## DISCUSSION

This series of greenhouse and field experiments showed indications of an effect of added Zn on reducing the levels of B-ODAP, or the toxin that causes lathyrism, in

**Table 6.** Concentration of the toxin B-ODAP in grain, hay and straw of grasspea lines in relation to added zinc (2001-2002).

| Zinc<br>kg ha <sup>-1</sup> | L.S 190 | L.S 288 | L.S 390 | L.S 716 | L.S 722 | L.S 736 | L.S 502 | L.S 670 | L.S 683 |
|-----------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| -----B-ODAP%, Grain-----    |         |         |         |         |         |         |         |         |         |
| 0                           | 0.26    | 0.21    | 0.28    | 0.35    | 0.38    | 0.39    | 0.66    | 0.54    | 0.65    |
| 5                           | 0.26    | 0.20    | 0.27    | 0.33    | 0.36    | 0.37    | 0.62    | 0.51    | 0.61    |
| 10                          | 0.24    | 0.18    | 0.25    | 0.30    | 0.33    | 0.33    | 0.56    | 0.46    | 0.55    |
| 20                          | 0.20    | 0.15    | 0.21    | 0.25    | 0.28    | 0.28    | 0.48    | 0.39    | 0.47    |
| Mean                        | 0.24    | 0.19    | 0.25    | 0.31    | 0.34    | 0.34    | 0.58    | 0.47    | 0.57    |
| SE±                         | 0.017   | 0.013   | 0.018   | 0.021   | 0.023   | 0.024   | 0.040   | 0.033   | 0.03    |
| -----B-ODAP%, Hay-----      |         |         |         |         |         |         |         |         |         |
| 0                           | 1.59    | 1.65    | 1.12    | 1.62    | 1.52    | 2.34    | 2.17    | 1.19    | 1.78    |
| 5                           | 1.63    | 1.70    | 1.23    | 1.02    | 1.92    | 1.16    | 1.69    | 2.33    | 1.60    |
| 10                          | 1.37    | 1.25    | 1.39    | 1.20    | 1.44    | 1.05    | 1.97    | 1.83    | 0.90    |
| 20                          | 1.55    | 1.49    | 1.45    | 1.57    | 1.85    | 2.00    | 1.78    | 1.90    | 1.58    |
| Mean                        | 1.53    | 1.52    | 1.30    | 1.35    | 1.68    | 1.64    | 1.90    | 1.81    | 1.46    |
| SE±                         | 0.12    | 0.20    | 0.15    | 0.29    | 0.24    | 0.63    | 0.21    | 0.47    | 0.39    |
| -----B-ODAP %, Straw-----   |         |         |         |         |         |         |         |         |         |
| 0                           | 0.32    | 0.35    | 0.32    | 0.30    | 0.38    | 0.26    | 0.33    | 0.28    | 0.33    |
| 5                           | 0.32    | 0.33    | 0.24    | 0.26    | 0.31    | 0.41    | 0.28    | 0.29    | 0.43    |
| 10                          | 0.35    | 0.29    | 0.36    | 0.32    | 0.27    | 0.32    | 0.27    | 0.23    | 0.28    |
| 20                          | 0.27    | 0.27    | 0.27    | 0.39    | 0.30    | 0.33    | 0.31    | 0.42    | 0.34    |
| Mean                        | 0.31    | 0.31    | 0.31    | 0.32    | 0.31    | 0.33    | 0.30    | 0.30    | 0.34    |
| SE±                         | 0.03    | 0.03    | 0.04    | 0.05    | 0.05    | 0.06    | 0.03    | 0.08    | 0.07    |

There were no significant effects.

the grain of a range of grasspea lines of varying innate levels of B-ODAP and of different parentage. The Zn effect appeared to be in addition to stimulating yield increases under conditions of Zn deficiency for the crop. Despite the inconsistency between varieties grown in the greenhouse and the field and between years in the field, there were consistent observations regarding Zn and the toxin. The decreases in B-ODAP with added Zn that we observed may be due to a chelating effect of the Zn on B-ODAP reducing its mobility to the grain.

Various studies from Mediterranean environments, such as in Australia (Hanbury *et al.*, 1999) and in Western Asia and North Africa (Abd El-Moneim *et al.*, 2000; 2001), where forage legumes are widely adapted, showed that B-ODAP varied widely in grasspea but was little influenced by the environment. However, the variation in B-ODAP in these grasspea lines over the three years of our trial where rainfall varied substantially would suggest otherwise. The screening program at ICARDA (Abd El-

Moneim *et al.*, 2000) showed that germplasm from Ethiopia, India and the sub-continent was generally high in B-ODAP (0.7% to 2.4%), with lines originating from the Near East having significantly lower toxin levels (0.02 to 1.2%). Using biotechnological techniques to develop toxin-free lines of *L. sativus*, Abd El-Moneim *et al.* (2001) exploited somoclonal variation to produce grasspea lines that are less than 0.1% B-ODAP but with many of the favourable agronomic traits retained, such as high yields and drought and disease resistance.

As Zn is an essential micronutrient for crop production, as was demonstrated in the greenhouse (Materon and Ryan, 1995) and field (Materon and Ryan, 1996), our study shows that in addition to providing adequate Zn nutrition for acceptable yields of grasspea, the Zn, either from the soil or applied as a fertilizer, may have the additional benefit of partially reducing B-ODAP levels in grasspea and thus making consumption of the crop safer for humans.



However, while increasing soil Zn may influence B-ODAP in grasspea grain, it is conceded that alternative approaches are also needed. Given that levels of B-ODAP above 0.2% are not considered safe for human consumption (Dahiya, 1976), and that applied Zn beyond the crop growth's needs had only a partial effect in reducing the grain B-ODAP concentration, the ultimate solution to lathyrism has to lie in varietal development through breeding low-toxin or zero-toxin *lathyrus* lines rather than soil fertilization, thus allowing this drought-resistant crop safely to occupy a niche role in human nutrition in harsh environments. Nevertheless, the subject of Zn in relation to B-ODAP leaves many scientific questions unanswered.

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## تأثیر مصرف کود روی در اصلاح مسمومیت (نئوروکسین) در گیاه خلر

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### چکیده

خلر (*Lathyrus sativus*) یک لگوم علوفه‌ای غنی از پروتئین بوده ولی محتوی یک نوع سم نئوروکسین (beta-ODAP) است که در صورت مصرف فراوان آن، سبب بروز ناهنجاریهای شدید در بدن می‌گردد. امروزه ارقام زیادی که محتوی مقدار کم این سم (پایین تر از ۲/۰ حد بحرانی) باشد متخصصین اصلاح نباتات تولید نموده‌اند، که این شواهد نشان می‌دهد که با مصرف روی، غلظت این سم را می‌توان به مقدار قابل توجهی کاهش داد. برای اثبات نقش روی در کاهش میزان سم B-ODAP، خاک‌های با غلظت پائین روی (۴۰ میلی گرم در کیلوگرم با روش DTPA) که از شمال سوریه تهیه شده بود، انتخاب و ارقام مختلف خلر در آن در دو آزمایش گلخانه‌ای و شرایط مزرعه-



ای-کودی کشت گردید. در حالی که در آزمایش اول گلخانه‌ای، نقش کودهای محتوی روی در کاهش سم B-ODAP در سه رقم خلر بسیار مثبت بود، کیفی در آزمایش دوم گلخانه‌ای، کاهش در مقدار سم (B-ODAP) موجود در ارقام با میزان سم بالا اتفاق نیافتاد. ولی از طرف دیگر فسفر اضافه شده نیز نقشی در میزان روی نداشت. در آزمایش مزرعه‌ای برای سه فصل با ۲۶۰، ۴۲۹ و ۴۰۵ میلی‌متر بارندگی، در ۹ رقم خلر، اضافه کردن مقادیر مختلف روی (۰، ۵، ۱۰ و ۲۰ کیلوگرم در هکتار) توانست مقدار سم B-ODAP را به مقدار ۱۰ الی ۴۰ درصد کاهش دهد. رابطه مستقیمی بین غلظت روی در دانه و کاه خلر با کاهش سم مشاهده نگردید. مکانیسم عمل که چرا با اضافه کردن روی، از میزان سم B-ODAP کاهش می‌یابد مشخص نیست. تحت شرایط مدیریت بهینه زراعی اثر روی در کاهش سم B-ODAP بخشی از راه حل برای کاهش سم B-ODAP می‌باشد. لیکن تولید ارقام با مقدار سم پائین بطوریکه اثرات سوئی در بدن نداشته باشد، توسط متخصصین اصلاح نباتات مورد نیاز می‌باشد.