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# Seed analysis as a means of identifying micronutrient deficiencies of *Phaseolus vulgaris* L. in the tropics

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**Seed analysis can indicate differences in micronutrient availability between soils. Comparison of *Phaseolus vulgaris* L. with published analytical data indicated that the micronutrients most limiting for N<sub>2</sub>-fixation and growth in parts of East and southern Africa were Mo and Cu. Significant differences in seed Mo concentration occurred between neighbouring farms. Less variation was found for Zn and Cu. Zinc and Cu in seed were inversely related to seed size and correlated with N, while Mo showed a significant inverse relationship to Zn and N but no relationship with seed size. A fivefold difference in Mo content occurred between two genotypes of similar seed size grown on the same fertile soil. Mean Mo content in the seeds of nine genotypes from a Mo-poor soil was 0.6 µg Mo seed<sup>-1</sup> and on a Mo-rich soil 6.4 µg Mo seed<sup>-1</sup>. None of the genotypes was a consistently good accumulator of micronutrients at all sites, and soil type was the major cause of differences between the sites. Sowing seeds of sufficient Mo content on a Mo-poor soil prevented the production of Mo-deficient seeds until the fourth consecutive harvest on the same site.**

Keywords: *Phaseolus vulgaris*; Seed analysis; Micronutrient deficiency; N-fixation

The common bean (*Phaseolus vulgaris* L.) is often grown in upland areas of the tropics where soils tend to be deeply weathered, strongly leached, and acidic with poor micronutrient availability. Several micronutrients are essential for N<sub>2</sub>-fixation, and the seed reserve can be a major source for nodulation and N<sub>2</sub>-fixation when external supplies are limiting.

Seed analysis is an alternative for the diagnosis of micronutrient problems not readily detected visually while analysis of a tissue sample, within the same season, may not leave sufficient time for correction of the deficiency within the same crop. Although visual deficiency symptoms may not appear, micronutrients may be at such low concentrations that N<sub>2</sub>-fixation is limited and yield reduced, as reported for Mo

(Doerge *et al.*, 1985), Co (Robson and Snowball, 1987), and Zn (Johnson and Simons, 1979). Information is available on the role of seed reserves of micronutrients (Hashimoto and Yamasaki, 1976; Robson and Mead, 1980) in supplying the requirements for growth and N<sub>2</sub>-fixation. Reports are available on seed micronutrient content as a useful indicator of deficiencies of micronutrients, e.g., Mo (Lavy and Barber, 1963; Franco and Munns, 1981; Ishizuka, 1982), Zn (Hallmark *et al.*, 1985), and Cu (Russ, 1958) in crops or in the soil from which they were harvested (Table 1).

The objectives of this study were (1) to analyse the concentration and amount of micronutrients in seeds to determine if these could be used in identifying micronutrients limiting N<sub>2</sub>-fixation and growth of *Phaseolus*; (2) to assess seed concentrations and contents from surveys on both a regional and a farm scale; and (3) to conduct field trials to investigate the relative roles of soil type and plant genotype in determining seed micronutrient content and the influence these might have on the use of seed analysis as a diagnostic tool.

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**Table 1** Critical concentrations of micronutrients in shoots and nodules, and critical amounts in seeds of *P. vulgaris* and other legumes as published in the literature

| Micronutrient | Status     | Shoots ( $\mu\text{g g}^{-1}$ ) | Seeds ( $\mu\text{g seed}^{-1}$ )   | Nodules ( $\mu\text{g g}^{-1}$ ) |
|---------------|------------|---------------------------------|-------------------------------------|----------------------------------|
| Mo            | Deficient  | <0.1 <sup>a</sup>               | 0.08 <sup>c</sup>                   | <3.6 <sup>d</sup>                |
|               | Sufficient | >0.5 <sup>a</sup>               | 3.5 <sup>b</sup> , 0.5 <sup>c</sup> | 3-6 <sup>b</sup>                 |
|               | Toxic      | >1500 <sup>i</sup>              |                                     |                                  |
| B             | Deficient  | 12-13 <sup>e</sup>              | 0.4-1.0                             |                                  |
|               | Sufficient | 26 <sup>e</sup>                 | >1.0                                |                                  |
|               | Toxic      | >200 <sup>e</sup>               |                                     |                                  |
| Zn            | Deficient  | <20 <sup>f</sup>                | <3-9 <sup>g</sup>                   |                                  |
|               | Sufficient | 40-50 <sup>f</sup>              | >10 <sup>g</sup>                    |                                  |
|               | Toxic      | >400 <sup>f</sup>               |                                     |                                  |
| Cu            | Deficient  | <4 <sup>h</sup>                 | <2 <sup>h</sup>                     |                                  |
|               | Sufficient | 15-25 <sup>f</sup>              | >4 <sup>h</sup>                     |                                  |
|               | Toxic      | >25 <sup>f</sup>                |                                     |                                  |
| Co            | Deficient  |                                 | 0.01 <sup>i</sup>                   |                                  |
|               | Sufficient |                                 | 0.73 <sup>i</sup>                   |                                  |
| Fe            | Deficient  | <10-30 <sup>j</sup>             | <10                                 |                                  |
|               | Sufficient | 100-800 <sup>f</sup>            | 20-150                              |                                  |
|               | Toxic      | >800 <sup>f</sup>               |                                     |                                  |
| Ni            | Deficient  |                                 | 0.010 <sup>k</sup>                  |                                  |
|               | Sufficient |                                 | 0.053 <sup>k</sup>                  |                                  |
| Mn            | Deficient  | <30 <sup>f</sup>                |                                     |                                  |
|               | Sufficient | 75-200 <sup>f</sup>             |                                     |                                  |
|               | Toxic      | >500 <sup>f</sup>               |                                     |                                  |

<sup>a</sup>Benton-Jones (1972); <sup>b</sup>Jacob-Neto and Franco (1986); <sup>c</sup>Meagher et al. (1952); <sup>d</sup>Jacob-Neto and Franco (1989); <sup>e</sup>Gupta and Cutcliffe (1984); <sup>f</sup>Shwartz and Gálvez (1980); <sup>g</sup>Hallmark et al. (1985); <sup>h</sup>Russ (1958); <sup>i</sup>Robson and Mead (1980); <sup>j</sup>Shorrocks (1983); <sup>k</sup>Eskew et al. (1983); <sup>l</sup>Mengel and Kirkby (1982)

\*Recognized not to be a good indicator of plant cobalt status

## Materials and Methods

### Surveys of micronutrients in plant material

Two surveys of micronutrients in plant materials of *P. vulgaris* were conducted. In the first

crops were sampled on a transect through the main bean-producing areas of northern, central, and southern Tanzania and northern areas of Zambia and Malawi to establish the ranges of concentrations of Mo, B, Zn, Cu, Co, Fe, Ni, and Mn in leaves and seeds and to identify those potentially limiting growth and N<sub>2</sub>-fixation. Leaves from 21 farms were sampled and seeds were collected from three regional markets and one research farm. In the second survey, seeds (about 200 g) of eight genotypes were collected from 27 farmers' fields in the Northern Province of Zambia and analysed for Mo, B, Zn, Cu, Co, Fe, Ni, and Mn to examine the effects of genotype and environment (and their interactions) on the micronutrient content of bean seed. Not all genotypes could be sampled in every field but they were all of fairly similar seed size.

### Field experiments

#### Experiment 1

Seeds of 18 genotypes of contrasting seed size (0.196-0.525 g seed<sup>-1</sup>) were sown in a randomised block design with four replicates on a fertile soil in Colombia (Table 2) to examine the relationships among seed micronutrient content, seed size, and genotype. The N, Cu, Zn, and Mo contents of ground sub-samples (about 200 g) of harvested seeds were determined.

#### Experiment 2

Studies on nine genotypes of *Phaseolus* were conducted at three sites of contrasting soil types (Table 2) in northern Tanzania to determine the contributions of genotype and soil to seed micronutrient content. The experiment at each field site was laid out as a randomised complete block design with three replicates. All plants received basal P at a rate of 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and micronutrient fertilizers were not applied. The seeds sown had been harvested from one location to reduce possible effects of differences in initial seed micronutrient content. Seeds harvested from one replicate of each genotype at each site were analysed for B, Fe,

**Table 2** Soil characteristics of the experimental sites in northern Tanzania and Colombia where field experiments were conducted

| Site              | Altitude (m) | Classification USDA  | pH  | Organic matter (%) | CEC (cmol kg <sup>-1</sup> ) | Base saturation (%) |
|-------------------|--------------|----------------------|-----|--------------------|------------------------------|---------------------|
| Northern Tanzania |              |                      |     |                    |                              |                     |
| Lambo             | 1020         | —*                   | 6.5 | 4.2                | 29                           | 75                  |
| Selian            | 1300         | Cumulic Haplustoll   | 6.6 | 3.7                | 41                           | 98                  |
| Lyamungu          | 1270         | —*                   | 5.4 | 4.7                | 31                           | 43                  |
| Colombia          |              |                      |     |                    |                              |                     |
| Quilichao         | 990          | Plinthidic Kandiodox | 5.7 | 4.3                | 25                           | —                   |
| Popayan           | 1700         | Typic Dystrandept    | 6.1 | 22.4               | 50                           | —                   |
| Restrepo          | 1350         | Typic Dystrochrept   | 6.6 | 4.8                | 37                           | —                   |
| Palmira           | 1000         | Aquic Haphidoll      | 7.0 | 2.4                | 12                           | —                   |

Both of these soils are Mollic Andosols under the FAO/Unesco classification system

Mn, Ni, and Co concentrations to observe differences among sites. Seeds from the three replicate plots of each genotype at each site were analysed for Zn, Cu, and Mo concentrations to examine differences among genotypes and among sites.

### Experiment 3

At three sites in Colombia with contrasting soil types (Table 2) field trials were carried out over four growing seasons ('87A, '87B, '88A, and '88B) to compare the seed micronutrients of four genotypes (BAT1297, Canadian Wonder, Kabanima, and Baseka). The genotypes Kabanima and Baseka were selected because they had shown evidence of being good and poor Mo accumulators, respectively, in Experiment 1. The experiment was laid out as randomised complete blocks with four replicates at each site. The seeds sown in '87A had been harvested from one location. Seeds from each subsequent harvest were replanted at the same site to begin the next season's field trial. Seeds from each trial were bulked and the estimates of Zn, Cu, and Mo concentration and content of the seeds sown and harvested were made from duplicate 1-g sub-samples from each seed lot.

### Chemical analysis

Samples of 100 g seed and at least 10 g leaves were rinsed with dilute nitric acid and distilled water before drying at 80°C for 48 h. Duplicate 1-g milled sub-samples were digested in nitric acid in sealed containers (Williams, 1978). Filtered digests were analysed for Zn and Cu by atomic absorption spectrometry (AAS); for Mo by graphite furnace AAS; and for B, Co, Fe, Ni, and Mn by inductively coupled plasma emission spectroscopy. The accuracy of the analysis was established using standard reference material (National Bureau of Standards, Canada). Total N contents were estimated by an indophenol blue method after semi-micro Kjeldahl analysis.

## Results

### General surveys

A comparison of micronutrient concentration in leaves and amount in seeds from the exploratory field survey with published critical concentrations and amounts in *Phaseolus* and other legumes (Table 1) showed that the availability of B, Co, Fe, and Ni was likely to be sufficient at all sites since no concentration or content in the plant materials was less than the critical values (Table 3). Zinc concentrations in plant shoots were low at three sites in Tanzania, and Mn was low at one site (Selian). Zinc was deficient in seeds of Small Buff and Ugogo genotypes from Moshi and Lushoto markets. Copper

**Table 3** Ranges of micronutrient concentrations in shoots, and amounts in seed for samples of *Phaseolus* from bean-producing areas in Tanzania, northern Zambia, and northern Malawi observed in the preliminary survey with an indication of potential nutritional status

| Micronutrient | Leaves ( $\mu\text{g g}^{-1}$ ) | Nutritional status | Seeds ( $\mu\text{g seed}^{-1}$ ) | Nutritional status |
|---------------|---------------------------------|--------------------|-----------------------------------|--------------------|
| Mo            | 0.3-5.2                         | S                  | <0.002-9.3                        | D to S             |
| B             | 30-127                          | S                  | 1.6-9.1                           | S                  |
| Zn            | 20-51                           | D to S             | 3.8-17.1                          | D to S             |
| Cu            | 1.7-13.1                        | D to S             | 1.3-4.5                           | D to S             |
| Co            | 0.10-1.6                        |                    | 0.02-0.15                         | S                  |
| Fe            | 168-1763                        | S to T             | 13-17                             | S                  |
| Ni            | 0.37-2.71                       |                    | 0.06-0.58                         | S                  |
| Mn            | 24-629                          | D to T             | 2.2-10                            | —                  |

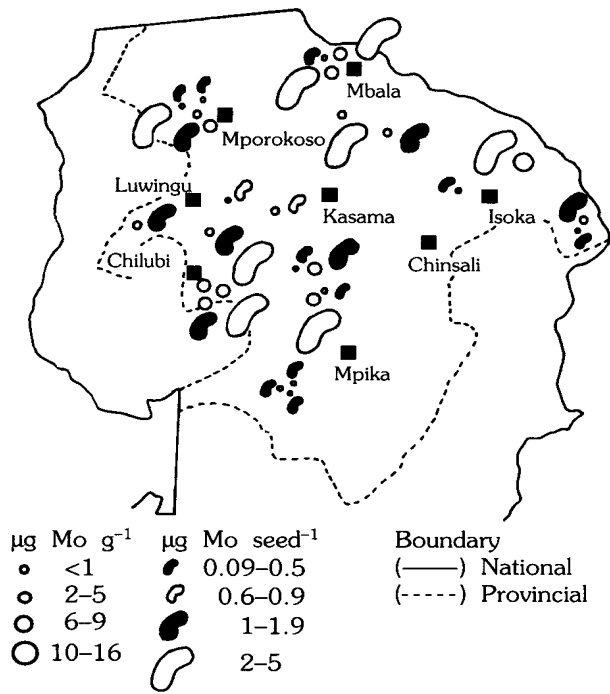
S, sufficient; D, deficient; T, toxic

and Mo concentrations and contents in seeds were frequently below the critical values and were deficient at all the sites sampled in Tanzania and Zambia. Large amounts of Fe and Mn were found in both shoots and seeds at Lambo in Tanzania and Mbala in Zambia, indicating that toxicity may have occurred at these locations. The widely differing Mo content of seeds obtained from the same market (0.7-9  $\mu\text{g Mo seed}^{-1}$ ) suggested that soil Mo status may vary considerably over relatively small areas. The full results of this survey are given in Brodrick (1990).

### Second survey, northern Zambia

Molybdenum concentrations ranged from 0.3 to 16  $\mu\text{g Mo g}^{-1}$  and contents from 0.09 to 5  $\mu\text{g Mo seed}^{-1}$ . Seeds of low Mo content (0.09-0.9  $\mu\text{g Mo seed}^{-1}$ ) occurred in all regions. Occasionally, a high Mo seed content was evident, e.g., in the genotype Kabanima sampled in the Isoka region (5  $\mu\text{g Mo seed}^{-1}$ , Figure 1). The soils in this part of northern Zambia are extremely acid (pH 3.9-4.5) and Mo is likely to be fixed in the soil and be of low availability to plants. Copper concentrations ( $7 \pm 0.1$  and range of 4-10  $\mu\text{g g}^{-1}$ ) were close to critical deficiency levels (4  $\mu\text{g Cu g}^{-1}$ ; 2  $\mu\text{g Cu seed}^{-1}$ ) in all samples suggesting that Cu deficiency was widespread. Boron contents (0.4-1.0  $\mu\text{g B seed}^{-1}$ ) were lower than had been seen in the preliminary micronutrient survey (1.6-9.1  $\mu\text{g B seed}^{-1}$ ) but were unlikely to be deficient. Iron and Mn content tended to be variable (range 12-62  $\mu\text{g Fe seed}^{-1}$  and 3-13  $\mu\text{g Mn seed}^{-1}$ ) with high contents at some sites, e.g., a genotype from Mukatula village in Mbala region contained 62  $\mu\text{g Fe seed}^{-1}$  and 9.1  $\mu\text{g Mn seed}^{-1}$ . Zinc contents (mean  $30 \pm 0.1$  and range of 16-40  $\mu\text{g g}^{-1}$ ) were above critical deficiency levels in all samples (<6  $\mu\text{g g}^{-1}$  or >3-9  $\mu\text{g Zn seed}^{-1}$ , Table 1) and showed less variation between genotypes and regions than Mo.

Samples from the same village showed a wide variation in Mo concentration between dif-

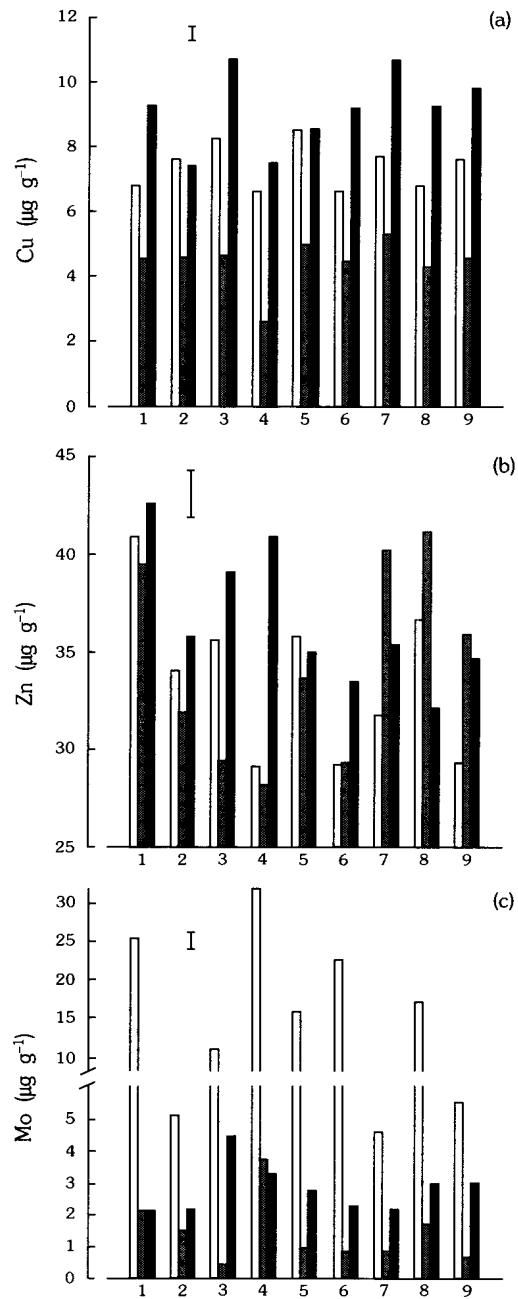


**Figure 1** Concentration and content of molybdenum in seed of *Phaseolus* collected from subsistence farmers in the Northern Province of Zambia

ferent genotypes. For example, at Mulyeko village in Mpika region Mo concentration was 6.3 µg Mo g<sup>-1</sup> in Namwikala genotype (small seed 0.205 g) and 0.7 µg Mo g<sup>-1</sup> in Mafi Yambusa genotype (large seed 0.427 g). However, no genotype was a consistent accumulator of Mo at all the sites sampled. The Chilubi district, on the banks of Lake Bangweuleu, consistently produced seeds of high Mo concentrations irrespective of genotype (>6 µg Mo g<sup>-1</sup>, Figure 1). Concentrations varied widely in all other regions (e.g., Mpika 0.28 µg Mo g<sup>-1</sup> to 7.56 µg Mo g<sup>-1</sup>). The indication that soil type and soil availability of micronutrients had a stronger effect on seed concentration and content of micronutrients than genotype was evaluated in experiments 2 and 3.

### Experiment 1

Seed weights of 18 genotypes of *Phaseolus*, grown on fertile soil at Palmira in Colombia, ranged from 0.195 to 0.525 g dry weight per seed (data not presented, see Brodrick, 1990). There was substantial variation in Mo content between two genotypes of similar seed size (Kabanima, 10.9 µg Mo seed<sup>-1</sup> and Baseka, 1.9 µg Mo seed<sup>-1</sup>) while contents of N, Cu, and Zn were more uniform between seeds of similar size. Molybdenum and N concentration showed no significant relationship with seed size ( $r = -0.49$  and  $-0.67$ , respectively;  $n = 18$ ,  $P > 0.05$ ). Zinc and Cu concentrations were positively correlated with N concentration ( $r = 0.863$  and  $0.744$ , respectively;  $n = 18$ ,  $P < 0.05$ ). Molybdenum exhibited a significant inverse correlation with N and Zn ( $r = -0.608$



**Figure 2** Concentration of (a) copper, (b) zinc, and (c) molybdenum in seed of nine genotypes of *Phaseolus* grown on three soils: Selian (□); Lambo (▒); Lyamungu (■) in northern Tanzania. Genotypes were 1, SD79/381; 2, Lyamungu 85; 3, Canadian Wonder; 4, TB79/420; 5, Kabanima; 6, MAK2-3; 7, Masai Red; 8, BAC66; and 9, EMP86. Vertical bars represent standard errors of the means

and  $-0.626$ , respectively;  $n = 18$ ,  $P < 0.05$ ) but was not correlated with Cu concentration ( $r = -0.146$ ;  $n = 18$ ,  $P > 0.05$ ).

### Experiment 2

Seeds of nine genotypes of *Phaseolus* grown in the same soil were planted in three contrasting soils in northern Tanzania. There were no dif-

ferences in the total amounts of B, Fe, Ni, and Mn in the seeds of the nine genotypes on the three contrasting soils ( $P > 0.05$ , data not presented). There was more Co in seeds from Lyamungu ( $0.3 \mu\text{g seed}^{-1}$ ) than from Selian ( $0.05 \mu\text{g seed}^{-1}$ ) or Lambo ( $0.07 \mu\text{g seed}^{-1}$ ;  $P < 0.05$ ). Seed concentration of Mn was higher at Lambo and Lyamungu ( $16 \mu\text{g g}^{-1}$ ) than at Selian ( $12 \mu\text{g g}^{-1}$ ;  $P < 0.05$ ), and Ni concentration was lowest at Selian ( $0.4 \mu\text{g g}^{-1}$ ), higher at Lambo ( $0.6 \mu\text{g g}^{-1}$ ), and highest at Lyamungu ( $0.8 \mu\text{g g}^{-1}$ ;  $P < 0.05$ ). The differences observed in concentration were masked by the range of seed sizes of the genotypes ( $0.16\text{--}0.69 \text{ g seed}^{-1}$ ) which increased the variation in seed content of micronutrients at each site, emphasizing the need to examine both concentration and content of micronutrients in seeds of genotypes with contrasting seed size.

Seed Cu concentration was smaller in seeds of all genotypes at Lambo than at Selian and Lyamungu ( $P < 0.05$ , Figure 2a). The concentration of Cu in the genotypes SD79/381, Canadian Wonder, MAK2-3, and Masai Red was greater at Lyamungu than at Selian ( $P < 0.05$ ). This was probably because the Lyamungu site was formerly a coffee plantation where large applications of Cu fungicides had been made. No single genotype consistently accumulated more Cu than the others at all sites. Therefore soil type and micronutrient availability were more important than genotype in determining high concentrations of Cu in seed on these soils. However, TB79/420 consistently had the lowest concentration at all three sites and therefore this genotype may not be able to absorb Cu from the soil or translocate it into the seeds as effectively as the other genotypes.

In the case of Zn, seed concentrations were similar at Lambo and Selian but were slightly higher overall at Lyamungu ( $P < 0.05$ , Figure 2b). Concentrations of Zn in some genotypes were significantly different from others but the differences were not consistent at all sites so that no genotype could be identified as a good accumulator of Zn.

Molybdenum concentrations were highest at Selian (mean =  $16 \pm 0.7 \mu\text{g Mo g}^{-1}$ ;  $P < 0.05$ , Figure 2c) where seed concentration was 10 to 20 times that required for symbiotic  $\text{N}_2$ -fixation ( $1\text{--}2.5 \mu\text{g Mo g}^{-1}$  depending on seed size, Meagher *et al.*, 1952). Concentrations were lower at Lyamungu (mean =  $3 \pm 0.7 \mu\text{g Mo g}^{-1}$ ;  $P < 0.05$ ) and lower still at Lambo (mean =  $1 \pm 0.7 \mu\text{g Mo g}^{-1}$ ;  $P < 0.05$ ). Seeds produced at Lambo are likely to be of deficient concentration and content. The cultivar TB79/420 had the highest concentration of Mo at Selian and second highest at Lambo and Lyamungu. There may be a relationship between Cu and Mo storage or uptake and translocation into the seed as the two micronutrients are known to interact antagonistically by competing at uptake sites (Olsen, 1972); Cu concentration was low in this genotype at all three sites.

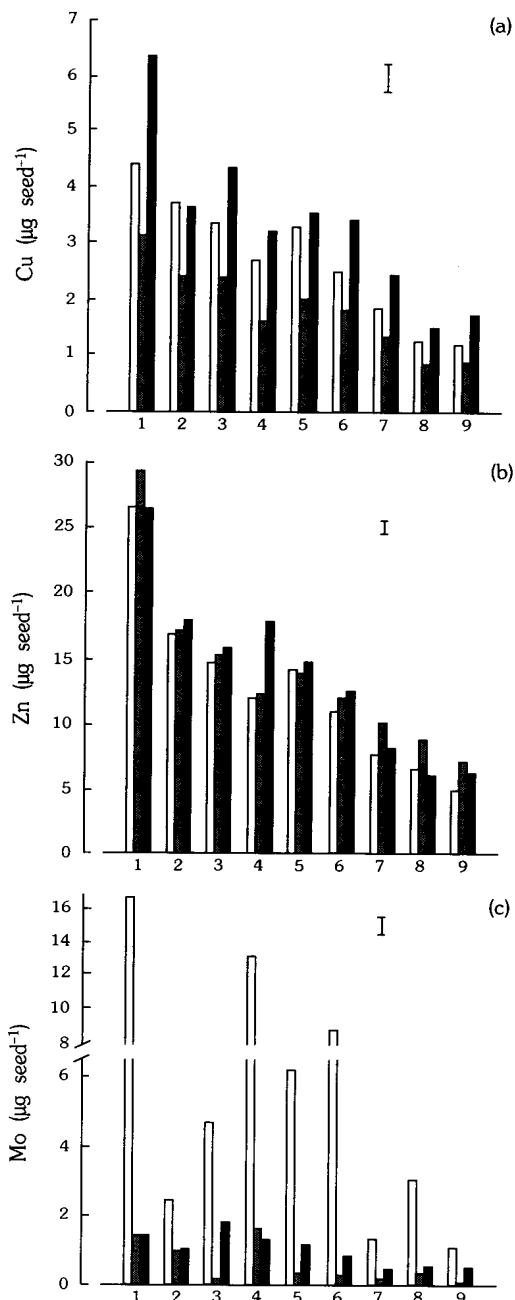
There was a severe drought at Lambo during this trial and therefore to ensure that the differences observed in Mo content in seed resulted from inherent Mo deficiency in the soil and not simply poor uptake due to lack of soil moisture, seed samples were taken in a subsequent season when rainfall was satisfactory. The results showed that contents in seed of the genotype Lyamungu 85 were significantly lower at Lambo ( $1.8 \mu\text{g Mo seed}^{-1}$ ) than at Selian ( $6.5 \mu\text{g Mo seed}^{-1}$ ).

Total amounts of Zn and Cu in seeds of the nine genotypes (Figure 3a and b) decreased with decreasing seed size. In contrast, the content of Mo showed no relationship with seed size (Figure 3c) confirming the results of seed analysis from Colombia. Genotypes of similar seed size varied substantially in their content of Mo, e.g., at Selian, Canadian Wonder and TB79/420 (both of seed weight  $0.41 \text{ g}$ ) had  $4.7 \mu\text{g Mo seed}^{-1}$  and  $13.2 \mu\text{g Mo seed}^{-1}$ , respectively. However, when the soil availability of a micronutrient was low all the seeds reserves were decreased dramatically, irrespective of seed size or genotype (i.e., Mo at Lambo). Mean Mo content in the seed of the nine genotypes at Lambo was  $0.6 \mu\text{g Mo seed}^{-1}$  and at Selian  $6.4 \mu\text{g Mo seed}^{-1}$ .

### Experiment 3

All contents of Cu and Zn at the three sites were above critical deficiency values of  $2 \mu\text{g Cu seed}^{-1}$  (Russ, 1958) and  $3\text{--}9 \mu\text{g Zn seed}^{-1}$  (Hallmark *et al.*, 1985; data not presented). This indicates that all three soils had good availability of Zn and Cu for *Phaseolus*. The small seed size of BAT1297 resulted in it having smaller contents of Zn and Cu than the three other larger-seeded genotypes ( $P < 0.05$ ).

Concentrations and contents of Mo in seed were lowest at Restrepo ( $3.2 \mu\text{g g}^{-1}$ ,  $1.2 \mu\text{g seed}^{-1}$ , respectively) and higher at Popayan and Quilichao ( $5.4 \mu\text{g g}^{-1}$ ,  $1.7 \mu\text{g seed}^{-1}$ , respectively; SEM = 0.72 and 0.22, respectively). The Mo content of Canadian Wonder, Kabanima, and Baseka seeds declined to amounts approaching the critical deficiency content of  $0.5 \mu\text{g seed}^{-1}$  by the fourth harvest at Restrepo (Table 4). Seed Mo content of BAT1297 was consistently low at all harvests at Restrepo. All the genotypes maintained sufficient contents of Mo in their seed in all seasons at Popayan and Quilichao (Table 4). The only exceptions were BAT1297 over the first two seasons at Quilichao, and Baseka in the last season at Popayan. Over all the sites and seasons Kabanima contained the highest amount of Mo in the seed ( $2.0 \mu\text{g seed}^{-1}$ ) followed by Canadian Wonder ( $1.9 \mu\text{g seed}^{-1}$ ), Baseka ( $1.3 \mu\text{g seed}^{-1}$ ), and BAT1297 ( $1.0 \mu\text{g seed}^{-1}$ ; SEM = 0.26). The Mo content of Kabanima seed was significantly higher than that of Baseka ( $P < 0.05$ ) despite their being of identical seed size ( $0.4 \text{ g seed}^{-1}$  dry weight).



**Figure 3** Content of (a) copper, (b) zinc, and (c) molybdenum in seed of nine genotypes of *Phaseolus* (ranked according to seed size) grown on three soils: Selian (□); Lambo (▒); Lyamungu (■) in northern Tanzania. Genotypes were 1, SD79/381; 2, Lyamungu 85; 3, Canadian Wonder; 4, TB79/420; 5, Kabanima; 6, MAK2-3; 7, Masai Red; 8, BAC66; and 9, EMP86. Vertical bars represent standard errors of the means

These data support the authors' observations in the Tanzanian field trials that poor soil availability of Mo is reflected in seed content, which is reduced to deficient amounts irrespective of genotype and their relative abilities to accumulate Mo in seed on other soils where it is available. Sowing seed of sufficient Mo content (Canadian Wonder, Kabanima, and Baseka) on

**Table 4** Molybdenum content of seed ( $\mu\text{g seed}^{-1}$ ) of four genotypes of *Phaseolus vulgaris* grown for four consecutive seasons at three sites in Colombia

| Genotype        | Site      | Molybdenum content of seed ( $\mu\text{g seed}^{-1}$ ) |     |            |     |     |
|-----------------|-----------|--|-----|------------|-----|-----|
|                 |           | Sown   |     | Harvested  |     |     |
|                 |           | 86   | 87A | 87B        | 88A | 88B |
| Canadian Wonder | Restrepo  | 1.7  | 0.4 | 0.8        | 0.9 | 0.6 |
|                 | Popayan   | 1.7  | 2.3 | 2.5        | 3.1 | 2.1 |
|                 | Quilichao | 1.7  | 3.5 | 3.9        | 0.6 | 2.2 |
| BAT1297         | Restrepo  | 0.4  | 0.5 | 0.5        | 0.6 | 0.3 |
|                 | Popayan   | 0.4  | 0.6 | 0.8        | 2.4 | 2.2 |
|                 | Quilichao | 0.4  | 0.2 | 0.4        | 0.8 | 3.0 |
| Kabanima        | Restrepo  | 4.5  | 4.0 | 1.0        | 1.7 | 0.6 |
|                 | Popayan   | 4.5  | 1.4 | 1.0        | 4.0 | 0.8 |
|                 | Quilichao | 4.5  | 2.8 | 1.0        | 4.2 | 1.4 |
| Baseka          | Restrepo  | 3.0  | 2.8 | 2.2        | 1.8 | 0.6 |
|                 | Popayan   | 3.0  | 1.7 | 1.6        | 0.7 | 0.4 |
|                 | Quilichao | 3.0  | 1.1 | 1.2        | 0.6 | 0.6 |
| SE              |           | $\pm 0.26$   |     | $\pm 0.99$ |     |     |

SE, standard error of the mean

a Mo-poor soil at Restrepo was able to prevent seed of deficient Mo content being produced until the fourth consecutive harvest of seed from the site.

## Discussion

### Surveys of micronutrients in plant material

The micronutrients most likely to be limiting  $\text{N}_2$ -fixation in the areas of Tanzania, Zambia, and Malawi from which samples were analysed were Mo and Cu. Zinc and Mn deficiency may occur at a few sites. Repeated sowing of beans in such soils can be expected to result in a depletion of seed reserves inadequate to support growth and  $\text{N}_2$ -fixation. Some leaf tissue analysis indicated potential Fe and Mn toxicity. In making these conclusions the authors are relying on the accuracy of published critical concentrations and contents of micronutrients.

Analysis of seed samples from markets in Tanzania had shown that, particularly in the case of Mo, it was possible to buy seed of widely differing micronutrient content in one market (Brodrick, 1990). This was reflected in the farm-to-farm differences in Mo content of the seed ( $0.72\text{--}6.26 \mu\text{g Mo g}^{-1}$ ) seen within the same village in northern Zambia. If seeds of a high micronutrient content were planted this might allay potential deficiencies. In addition, seed of low Mo content can have reduced viability thus reducing the per cent germination within a crop (Peterson and Purvis, 1961).

Seed content could be used to make predictions of micronutrient fertilizer requirements of soils. This is only valid, however, if other relevant soil conditions (e.g., moisture content, physical impediments to rooting) are not limiting nutrient uptake. Importing seed of high Mo content onto a Mo-deficient soil in northern Zambia improved N<sub>2</sub>-fixation and increased seed yield in *Phaseolus* (Brodrick *et al.*, 1992). In the Northern Province of Zambia one locality was identified as consistently producing seed of high Mo content (Chilubi). Such regions could be developed as producers of high Mo seed content. Redistribution of seed with a high Mo content might be feasible on a local basis. Seed reserves of Zn and Cu were less variable at a local level than those of Mo which confirms the greater variation in Mo content between soils and between genotypes which was observed in the field trials.

### Field experiments

Concentrations and contents of micronutrients in seed are controlled by genotype (seed size, accumulation into seed) and soil type on which the plants are grown. Concentrations of Zn and Cu were inversely related to seed size. Despite this, the smaller-seeded genotypes still had lower total amounts of Zn and Cu in their seeds. In the case of Mo, there was no obvious relationship between concentration and seed size; in fact, seeds of similar size had a fivefold difference in concentration and content between the genotypes Kabanima and Baseka grown on the same soil. This suggests that the ability to accumulate Mo into the seed varies quite considerably between genotypes while the ability to accumulate Zn and Cu is less variable. Genotype Kabanima was more efficient than Baseka in its utilization of Mo for N<sub>2</sub>-fixation and translocated a higher percentage of total plant Mo into the seed in controlled environment experiments (Brodrick and Giller, 1991).

When seed micronutrient content of a number of genotypes grown on soils of contrasting micronutrient availability was compared it was found that the soil exerted a stronger regulation on micronutrient content of seed than genotype (e.g., Mo at Lambo).

None of the genotypes tested in Tanzania was consistently a better accumulator of micronutrients into the seed than the others; however, it appeared that one genotype (TB79/420) was a poor accumulator of Cu. In Colombia sown seed of a high Mo content was decreased to near deficient amounts in three large-seeded genotypes after four seasons' growth in a soil (Restrepo) from which Mo was poorly available. Over all the sites and seasons Kabanima proved to be the best accumulator of Mo into the seed, but this did not prevent its seed contents from falling to deficient amounts in the Mo-poor soil at Restrepo.

Poor Mo availability in soil appears to override the effect of genotype and therefore it may not be necessary to identify genotypes which are good accumulators of Mo prior to using seed analysis as a means of diagnosing deficiency. Planting seed of high Mo content may prevent the production of Mo-deficient seed for several seasons. The contents of Zn and Cu in the seed harvested in Colombia did not vary between the three soils and remained similar to the content of the seed sown.

### Conclusion

Seed analysis is a sensitive indicator of soil availability of micronutrients, particularly in soils from which a micronutrient is poorly available. When interpreting results a number of factors must be taken into account such as seed size, genotype, previous land use, soil moisture status, and micronutrient content of the sown seed. If a soil is very poor in a micronutrient then the genotypic differences become less pronounced as the seed contents of all genotypes are decreased to deficient amounts.

Seed reserves may be used as a general measure of the extent of micronutrient deficiency in a province or to identify regions within the province as well as farms about a village where there is no micronutrient deficiency.

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