ZINC AVAILABILITY AFFECTED BY CULTIVATION CONDITIONS IN INDIAN MUSTARD *BRASSICA JUNCEA* CV RLM 198

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INTRODUCTION

Poor growth and poor nutrient accumulation of plants due to inadequate nutrient supply from the soil is a common phenomenon in many geographical areas. Almost one-fourth of the soils of the world have some kind of inherent mineral deficiency or toxicity problem (1). Deficiency symtoms in man for several trace elements have been traced back via animals and plants to inadequate element supply from the soil (2,3).

Zinc deficiency is one of the most common micronutrient deficiencies affecting agricultural crops in the United States (4). Today, at least 24 states recommend the addition of zinc to soil for certain crops (5). India is also known to have areas of zinc deficient soils which includes the major *Brassica* oil seed producing states (6).

With respect to zinc the relationship between deficiency in soil-plant-animal-man is unexplored. Factors that contribute to the occurence of zinc deficiency in man and lifestock are not well defined. However, it is obvious that inhibitors are predominant in human foods and animal feeds as well as in soils.

Several attempts have been made lately to identify the specific constituents of *Brassica* seeds involved in the metabolism of zinc in plant-animal-man (7,8,9). In these investigations cultivation conditions have been suggested to have a significant influence on the distribution of the seed constituents involved in zinc metabolism. For the small Indian farmer fertilizers are too costly and the existing soil conditions are therefor of major importance for the nutriture of the plant.

The present investigation was carried out to elucidate the possible influence of fertilizer and irregation on the metabolism of zinc in plant-animal- man. Bioavailability of native seed zinc and added zinc sulphate was estimated in growing weanling rat identified as a suitable animal model for zinc availability in foods and feeds (2).

MATERIAL AND METHODS

Seeds: Table 1 summerises cultivation conditions for five seed samples of Indian mustard *Brassica juncea* cv RLM 198. Seed batch No 1 was cultivated in India in 1979-80 under standard conditions. The obtained seeds were multiplied in Sweden in 1981 under standard conditions batch No 2. In 1980-81 another batch of seeds were cultivated in India excluding irregation, No 3, and fertilizer, No 4. Seed batch No 5 served as a control for No 3 and 4 having irregation and fertilizer included in the cultivation conditions.

Methods of meal preparation: Seeds were lyophilized and grind twice in a roller mill at 20°C. Oil was extracted at 20°C in hexane as described before (10). The deoiled seed meals were stored a few days at -20°C before mixing into diets.

Chemical analysis: Zinc content of deoiled seed meal and serum were analysed by atomic absorption spectroscopy after wet ashing in perchloric acid and hydrogen peroxide (1:2). Phytic acid was determined by colorimetry, essentially as described by Wheeler and Ferrel (11). Instead of 3% TCA, 0.5% H_2SO_4 was used for phytic acid extraction and precipitation. Zinc availability *in vitro* was assessed by enzymatic digestion at pH 1.35 and measurement of souluble zinc at pH 7.4 as described for Iron (12).

Bioassay: Weanling male rats of the Sprague-Dawley strain obtained from a commercial breedar (Anticimex, Sollentuna, Sweden) were housed in polypropylene cages with an open grill stainless steel floor. Lighting, humidity and temperature were controlled. Deionized water and food were given *ad libitum* for 7 days. Diets were formulated to contain 1.5, 3.0 and 4.5 ppm of zinc from seed meal or $ZnSO_4 \cdot 7H_2O$. Adequate amounts of other nutrients were incorporated in the diets as described elsewhere (13). Blood was obtained from the tail and linear correlation was calculated between serum zinc and dietary zinc. Percent available zinc was obtained from the ratio between slope of sample and slope of pure zinc sulphate.

Statistical analysis: Statistical evaluation of the slope-ratio assay was performed as described elsewhere (13). The significances of the differences of the means were determined by Student's t-test.

RESULTS AND DISCUSSION

Zinc content of seed samples varied between 75 and 104 ug/g in the deoiled seed meals (Table 2). The exclusion of fertilizer during cultivation of seed batch No 4 seemed to impaire the accumulation of zinc. Furthermore the zinc present in this meal had the lowest bioavailability. A wide range in availability up to 104% was present in the other seed samples.

The addition of equal amount of zinc sulphate (4.5 ppm) to equal amount of seed zinc (4.5 ppm) in the five seed meals gave rise to different response in serum zinc (Table 3). The highest availability was obtained for seed meal No 4 (lowest availability of native seed zinc). On the contrary zinc sulphate added to meal No 3 seemed virtualy unavailable. Seed meals derived from seeds cultivated at standard conditions, No 1,2 and 5, responded intermediate to the zinc sulphate supplementation.

The different response to fertilizer and irregation observed with respect to availability of native seed zinc as well as added zinc sulphate demonstrates the significance of cultivation conditions. These results indicates furthermore the presence of different seed constituents involved in the metabolism of native seed zinc and added zinc sulphate.

Phytic acid content of the different seed meals varied from 2.6% to 3.7% (Table 4). This variation was obviously related to cultivation conditions other than irregation and fertilizer in India. Although phytic acid has been demonstrated to interfere with zinc utilization in *Brassica* seed meals (9) no obvious relationship between phytic acid content and zinc availability was observed in this study. Other seed constituents seem to be the major determinants of zinc metabolism.

In vitro availability of zinc was generally lower than *in vivo* (Table 5). Pepsin digestion in HCl, simulating stomach conditions, desolved practically all seed zinc. Adjustment of pH to the neutral value of the intestine precipitated most of the solubilized zinc. The remaining soluble zinc at pH 7.4 is defined as available zinc. Seed batch No 2 and 1 have the highest values *in vivo* as well as *in vitro*. No 3, 4 and 5 are all low in both methods.

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Table 1.

Cultivation conditions of <u>Brassica juncea</u> cv RLM 198 in India and Sweden

Seed Batch	Sowing Country	Sowing Date	Nutrients (kg/ha) N	Nutrients (kg/ha) P_2O_5	Irregation
1	India[a]	Oct.15 1980	100[b]	50[c]	four
2	Sweden[d]	April 29 1981	60[c]	30[c]	-
3	India[a]	Oct.15 1981	100[b]	50[c]	none
4	India[a]	Oct.15 1981	no fertilizer		four
5	India[a]	Oct.15 1981	100[b]	50[c]	four

[a] sandy loam soil

[b] half applied at sowing time and half at flowering time

[c] applied at sowing time

[d] clay loam

Table 2.

Bioavailability of native zinc in defatted seed samples, feeding trials with weanling rats

Seed Batch	Cultivation conditions	Seed zinc ug/g	Bioavailable zinc ug/g	Bioavailable zinc %
1	India: standard cond.	74	61	82
2	Sweden: standard cond.	74	77	104
3	India: no irregation	92	49	53
4	no fertilizer	75	28	37
5	irregation fertilizer	104	64	62

Table 3.

Bioavailability of zinc sulphate added to native seed zinc; serum zinc in feeding trials with weanling rats

Seed Batch	Cultivation conditions	S E R U M Z I N C ug / ml Zinc source Seed Zn[a]	Seed Zn[a] +Zn sulphate[b]	Increase due to Zn sulphate
1	India: Standard cond.	0.77	0.98[c]	0.21
2	Sweden: Standard cond.	0.83	1.01[c]	0.18
3	India: no irregation	0.74	0.78	0.04
4	no fertilizer	0.59	1.22[c]	0.63
5	irregation fertilizer	0.77	1.02[c]	0.25

[a]Dietary seed zinc was 4.5 ppm

[b]Dietary zinc sulphate was 4.5 ppm

[c]Significant higher after Zn sulphate supplementation (p=0.001)

Table 4.

Phytic acid content of seeds and bioavailability of native seed zinc and added zinc sulphate

Seed Batch	Cultivation conditions	Phytic acid %	Bioavailable zinc seed Zn %	Zn sulphate serum Zn increase ug/ml
1	India: Standard cond.	2.6	82	0.21
2	Sweden: Standard cond.	3.7	104	0.18
3	India: no irregation	3.1	53	0.04
4	no fertilizer	3.1	37	0.63
5	irregation fertilizer	3.0	62	0.25

Table 5.

Availability of seed zinc estimated *in vivo* and *in vitro*

Seed Batch	Cultivation conditions	Availability of seed zinc In vivo %	In vitro Soluble zinc pH 1.35 %	pH 7.4 %
1	India: Standard cond.	82	86	7.8
2	Sweden: Standard cond.	104	85	13
3	India: no irregation	53	84	4.6
4	no fertilizer	37	83	5.3
5	irregation fertilizer	62	87	5.1

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