Soybean genetic differences in response to Fe and Mn: Activity of metalloenzymes

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Summary Metalloenzymes as biochemical markers of nutritional status have been studied for 30 years since Brown and Hendricks⁵ proposed the use of enzyme activities as indicators for the diagnosis of micronutrient deficiencies. Enzyme activities can be used in other lines of research, such as the characterization of particular reactions of genotypes to nutritive imbalances.

Catalase, peroxidase and superoxide dismutase activities were assayed in soybean cultivars growing in nutrient solutions with different Fe/Mn ratios. Considerable differences were observed between cultivars. Relationships between growth and enzyme activity were also studied.

Although more information is required, our results provide an introduction to the knowledge of the mechanisms developed by genotypes in response to Fe-Mn imbalances.

Introduction

Information about the specific reactions of plant genotypes to nutritional stress is the first approach to improve the efficiency of plant production under limiting conditions²¹.

Iron and manganese nutritional disorders are rather common in acid or alkaline soils. Early reports have indicated the existence of a certain Fe-Mn balance for optimum growth^{22,23} but the real importance of the Fe-Mn relationship remains unclear¹⁸, and the situation is complicated by the different responses of genotypes to Fe and Mn nutrition. Several authors have pointed out differences in terms of efficiency in iron absorption^{6,24} or in Mn use²⁰, as well as in tolerance to excess of Fe and Mn nutrient supplies^{3,7,8,17,20}.

The use of metalloenzymes as biochemical markers of plant nutrient status has the advantage of the high sensitivity and specificity of enzymatic analysis^{2,9}. The activities of the metalloenzymes catalase, peroxidase and superoxide dismutase were found to be related to Fe and Mn supply in soybean¹⁹ and in other species⁹, and thus could be used as indicators for the appraisal of Fe and Mn nutrient status.

In the present work, we analyze some metalloenzyme activities from soybean cultivars showing diverse responses to Fe and Mn nutrition in an attempt to characterize the different behaviours of the genotypes on a biochemical basis.

Materials and methods

Plant material

Soybean (*Glycine max* (L.) Merr.) cultivars with different response to Fe and Mn (Bragg, Lee, Forrest, T-203) were grown in aerated nutrient solutions with the following composition in meq/l: NO_3^- , 10; PO_4H_2^- , 0.5; SO_4^{2-} , 4; K^+ , 5; Ca^{2+} , 9; Mg^{2+} , 3; and in ppm: B, 0.5; Cu, 0.05; Zn, 0.05; Mo, 0.03. The initial pH was 5.5. Nine treatments were used varying Fe (Fe^0 : 0.25; Fe_1 : 1.0; Fe_2 : 2.5 ppm) (as Fe-EDDHA) and Mn (Mn_0 : 0.0; Mn_1 : 0.1; Mn_2 : 5.0 ppm). Plants were grown in a growth chamber, CONVIRON mod.PGW-36, under 14 h light and 10 h dark periods, at 30°C and 20°C, respectively. Photosynthetic active radiation was approximately $390 \mu\text{E} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$.

Enzyme assays

The first trifoliate leaf, at the second trifoliate stage (14 days old), was sampled for analysis. Catalase was determined polarographically by the oxygen electrode method¹⁰, peroxidase was estimated using o-dianisidine as H-donor¹¹ and superoxide dismutase by a photochemical method¹⁶. Mn-superoxide dismutase isozyme (Mn-SOD) was differentiated from total superoxide dismutase (Total-SOD) by performing the activity assay in the presence and in the absence of 1 mM NaCN.

Other determinations

Dry weight of the plant material was determined after 48 h in a forced draft oven at 70°C. Tissue Fe and Mn were determined by atomic absorption spectrophotometry.

Experimental design

A factorial design (3 Mn $\times$ 3 Fe levels) with three replications was used.

Results and discussion

Symptoms of Fe deficiency appeared on leaves of cultivar T-203 grown in solutions with low (0.25 ppm) or medium (1.0 ppm) Fe concentrations. In Bragg, Lee and Forrest, symptoms resembling Fe chlorosis and Mn toxicity (crinckle leaf) were observed on leaves of plants grown in solutions of 5.0 ppm Mn with 0.25 ppm Fe. Dry matter values of the first trifoliate leaves are shown in Table 1.

The catalase activity of the different soybean cultivars is shown in Fig. 1. Increases in Fe supply produced different effects with varying Mn concentrations. At low Mn supply, there was a general trend to an increase in catalase activity when Fe concentration was raised. However, at medium or high Mn concentrations, the response of catalase activity to increasing Fe supplies depended on the cultivars, remaining positive only in Forrest and T-203. At each Fe concentration, the increase in Mn supply determined a different behaviour for catalase activity in the cultivars. Only at the highest Fe level, the increase in Mn caused a general tendency (except in Forrest) to depress catalase activity. Previously, Agarwala *et al.*¹ had observed higher catalase activity at medium Mn supply than at lower or higher concentrations in the entire range of Fe supply. Other authors have shown a relation between catalase activity and Fe levels suggesting the possibility of using that

Table 1. Dry weights of first trifoliolate leaves of soybean cultivars grown with different Fe and Mn levels. Values followed by the same letter within columns are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test

Nutrient level (ppm)		Leaf growth (mg. leaf ⁻¹)			
Mn	Fe	Bragg	Lee	Forrest	T-203
0.0	0.25	95.5 de	78.4 b	59.0 b	118.5 d
0.0	1.0	112.4 bcd	75.9 b	91.7 a	115.6 d
0.0	2.5	117.9 bc	86.1 b	102.0 a	135.2 cd
0.1	0.25	141.8 a	111.6 a	109.9 a	109.1 d
0.1	1.0	122.2 abc	118.9 a	109.7 a	173.2 ab
0.1	2.5	130.5 ab	116.8 a	111.5 a	184.1 a
5.0	0.25	45.8 f	67.4 b	36.9 b	122.5 d
5.0	1.0	106.9 cd	81.9 b	99.5 a	116.4 d
5.0	2.5	86.4 e	79.8 b	102.9 a	150.2 bc
Significance of treatments differences ($P < \dots$)					
Mn		0.001	0.001	0.001	0.001
Fe		0.001	n.s.	0.001	0.001
Mn $\times$ Fe		0.001	n.s.	0.001	0.001

Fig. 1. Leaf catalase activity of soybean cultivars grown under different Fe and Mn levels.

enzyme as a functional indicator of the Fe involvement in plant metabolism processes^{9,11}. We could not observe a relationship between the leaf Fe concentration (Table 2) and the catalase activity whereas Mn concentration (Table 3) was correlated to the enzyme in Bragg (correlation

Table 2. Iron concentration in first trifoliolate leaves of soybean cultivars grown with different Fe and Mn levels. Values followed by the same letter within columns are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test

Nutrient level (ppm)		Iron concentration ($\mu\text{g.g dry wt.}^{-1}$).			
Mn	Fe	Bragg	Lee	Forrest	T-203
0.0	0.25	84.9 cd	74.5 e	95.1 cde	51.1 b
0.0	1.0	125.4 ab	142.7 a	96.7 cde	57.2 ab
0.0	2.5	114.3 ab	101.5 bcde	102.1 cd	80.1 a
0.1	0.25	89.8 cd	77.1 de	117.6 bc	81.9 a
0.1	1.0	129.9 a	107.2 bc	169.6 a	121.1 c
0.1	2.5	181.5 e	130.4 ab	135.4 ab	83.3 a
5.0	0.25	77.8 d	106.4 bc	133.3 ab	62.3 ab
5.0	1.0	99.8 bc	82.5 cde	78.1 e	57.9 ab
5.0	2.5	99.9 bc	84.2 cde	87.4 de	61.7 ab
Significance of treatments differences ($P < . . .$)					
Mn		0.001	n.s.	0.001	0.001
Fe		0.001	0.01	n.s.	n.s.
Mn $\times$ Fe		0.05	0.001	0.001	n.s.

Table 3. Manganese concentration in first trifoliolate leaves of soybean cultivars grown with different Fe and Mn levels. Values followed by the same letter within columns are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test

Nutrient level (ppm)		Manganese concentration ($\mu\text{g.g dry wt.}^{-1}$).			
Mn	Fe	Bragg	Lee	Forrest	T-203
0.0	0.25	15.7 b	18.5 a	14.8 a	12.3 c
0.0	1.0	14.3 b	10.9 b	14.0 a	9.5 d
0.0	2.5	12.3 c	14.3 c	16.2 a	12.6 c
0.1	0.25	56.8 a	69.2 d	58.8 bc	61.7 b
0.1	1.0	52.0 a	52.6 e	64.2 b	51.1 b
0.1	2.5	52.9 a	38.1 f	54.6 c	33.4 e
5.0	0.25	846.8 d	1179.4 g	70.6 d	285.6 a
5.0	1.0	624.1 e	868.9 h	665.1 d	472.7 f
5.0	2.5	324.8 f	407.2 i	389.8 e	291.1 a
Significance of treatments differences ($P \times . . .$)					
Mn		0.001	0.001	0.001	0.001
Fe		0.001	0.001	0.001	0.01
Mn $\times$ Fe		0.001	0.001	0.001	0.001

coefficient, $r = -0.497^{**}$), Lee ($r = -0.551^{**}$), T-203 ($r = -0.657^{***}$) and Forrest (parabolically, $y = 322.092 + 0.849.x - 0.001.x^2$, $F_c = 12.3$). The expression of catalase activity depended on various factors (Fe and Mn supply, cultivar assayed) but apparently there was a more direct relation of the enzyme activity with Fe supply in the Fe-inefficient cultivars (Forrest, T-203) (Fig. 1).

Manganese stress (deficiency or toxicity) increased peroxidase activity (Fig. 2) although individual responses of cultivars also were observed. Peroxidase activity was higher in the Mn-intolerant cultivars (Bragg,

Fig. 2. Leaf peroxidase activity of soybean cultivars grown under different Fe and Mn levels.

Forrest) under Mn-toxicity, while the same was detected for the Mn-tolerant cultivars (Lee, T-203) under Mn-deficiency (Mn₀). The increase in Fe supply at each Mn level failed to produce increases in the peroxidase activity as it was shown in other species^{2,9}. Foliar Fe concentration (Table 2) was negatively correlated to peroxidase activity in Bragg ($r = -0.446^*$) and T-203 ($r = -0.503^{**}$) cultivars. Leaf Mn concentration (Table 3) was positively related to the enzyme in Bragg ($r = 0.952^{***}$) and Forrest ($r = 0.920^{***}$), but negatively in T-203 ($r = -0.566^{**}$) cultivars.

An interesting point was the negative correlation between growth (Table 1) and peroxidase activity in the cultivars assayed (Bragg, $r = -0.810^{***}$; Lee, $r = -0.713^{***}$; Forrest, $r = -0.643^{***}$; T-203, $r = -0.581^{***}$) in agreement with those reports describing the involvement of peroxidases in plant growth processes¹⁵.

Treatments affected superoxide dismutase activities (Total-SOD and isozyme Mn-SOD) in a different manner (Tables 4 and 5). Total-SOD activity was always affected by Fe levels and in a variable way by Mn or Fe-Mn interactions, but on the contrary, Mn-SOD activity was only modified by Mn levels. Foliar Fe concentration was negatively correlated to Total-SOD activity in Bragg ($r = -0.513^{**}$) while Mn concentration was positively correlated to Mn-SOD activity in Bragg ($r = 0.635^{***}$), Lee ($r = 0.721^{***}$), Forrest ($r = 0.705^{***}$) and T-203 ($r = 0.625^{***}$). Previous works have indicated a dependence of Mn-SOD activity on the Mn nutrient supplies¹³ and the induction of a new Mn-SOD isozyme by high nutrient levels of Mn¹².

Table 4. Total-SOD activity in leaves of soybean cultivars grown with different Fe and Mn levels. Values followed by the same letter within columns are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test

Nutrient level (ppm)		Total-SOD activity (units.ml ⁻¹).			
Mn	Fe	Bragg	Lee	Forrest	T-203
0.0	0.25	39.05 ab	39.69 ab	39.57 ab	37.73 ab
0.0	1.0	37.67 b	41.56 ab	40.04 ab	40.83 ab
0.0	2.5	37.83 b	38.21 b	35.93 a	34.76 ac
0.1	0.25	44.90 c	44.38 c	40.82 ab	41.62 ab
0.1	1.0	38.03 b	38.52 b	39.13 ab	38.66 ab
0.1	2.5	38.89 ab	38.79 b	37.78 ab	30.50 c
5.0	0.25	50.46	42.94 ac	42.55 b	42.63 b
5.0	1.0	43.15 ac	44.79 c	42.43 b	43.35 b
5.0	2.5	37.88 b	38.17 b	38.51 ab	42.30 b
Significance of treatments differences ($P < . . .$)					
Mn		0.001	n.s.	n.s.	0.05
Fe		0.001	0.01	0.05	0.05
Mn $\times$ Fe		0.01	0.05	n.s.	n.s.

Table 5. Mn-SOD activity in leaves of soybean cultivars grown with different Fe and Mn levels. Values followed by the same letter within columns are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test

Nutrient level (ppm)		Mn-SOD activity (units.ml ⁻¹)			
Mn	Fe	Bragg	Lee	Forrest	T-203
0.0	0.25	20.94 a	25.58 ab	22.65 a	22.58 ab
0.0	1.0	18.49 a	23.04 a	22.36 a	20.39 a
0.0	2.5	21.79 a	27.83 abc	22.23 a	22.96 ab
0.1	0.25	37.64 b	36.19 cd	33.73 bc	26.25 ab
0.1	1.0	26.42 ac	29.43 abc	31.68 c	30.33 ab
0.1	2.5	30.93 bc	29.29 abc	29.07 ac	22.76 ab
5.0	0.25	34.16 bc	42.28 d	37.36 bc	29.41 ab
5.0	1.0	32.38 bc	36.19 cd	30.24 ac	35.05 b
5.0	2.5	33.95 bc	33.22 bcd	40.90 b	33.64 b
Significance of treatments differences ($P < . . .$)					
Mn		0.001	0.001	0.001	0.01
Fe		n.s.	n.s.	n.s.	n.s.
Mn $\times$ Fe		n.s.	n.s.	n.s.	n.s.

The correlation studies showed positive coefficients between Total-SOD and peroxidase activities in Bragg ($r = 0.705^{***}$) and Forrest ($r = 0.422^*$). A negative correlation was found between catalase and Mn-SOD activity in Lee ($r = -0.403^*$), or catalase and Total-SOD activity in T-203 ($r = -0.406^*$). Only in Lee cultivars's could a positive relation between catalase and peroxidase activities be registered ($r = 0.625^{***}$).

The enzymes studied, all involved in oxygen metabolism, were affected

in different ways by treatment in the cultivars. The conditions of deficiency and toxicity of each metal could generate stress situations by inducing the production of oxygen free radicals¹⁴. Active oxygen species such as metal-oxygen complexes could also be formed in certain biochemical systems from iron, copper and manganese²⁵. Brown and Devine⁴ indicated that the form of Mn (Mn^{4+} or Mn^{2+}) inside the plant might be a controlling factor of Mn tolerance. However, considering our results, other factors could be involved.

Whether the behaviour of soybean genotypes under metallic stresses could be related to the particular induction of protective enzymes (genetically coded) or to differential metabolic production of toxic radicals are problems that remain to be clarified in the future.

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