

# GENETIC DIFFERENCES IN THE ACCUMULATION OF MINERAL ELEMENTS IN SEEDS OF TROPICAL SOYBEANS *GLYCINE MAX* (L.) Merrill<sup>1</sup>

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**ABSTRACT** - The soybean is a major crop in the agricultural systems of the Brazilian Cerrados (Savannahs), whose soils are acidic, devoid of nutrients and need to be amended before they are cultivated. However, below the ploughed layer, there is scarcity of nutrients and toxic aluminium. These limit root growth, causing nutritional unbalance and drought stress. The mineral element content in the seeds may be negatively affected, with consequences to humans and livestock. The present work aimed to identify genetic differences of mineral element accumulation in the seeds of soybeans by a 9 x 9 diallel cross among adapted varieties, grown in high and in low-Al areas. The Covariance ( $W$ ) - Variance ( $V$ ) relationship indicated that the additive-dominance model explained the genetic differences in this germplasm for Ca, Mg, Al and Mn. For the elements P, K, Fe, Zn, Cu, Mo, Na and Ti, the genetic effects seemed more complex. The level of mineral elements was affected by Al and pH but did not compromise food quality. Additional studies are suggested to further clarify the genetics of mineral element accumulation in soybean seeds.

**Index terms:** acid soils, aluminium, toxicity, nutrient, variety, diallel cross.

## DIFERENÇAS GENÉTICAS NA ACUMULAÇÃO DE ELEMENTOS MINERAIS NA SEMENTE DE SOJA TROPICAL *GLYCINE MAX* (L.) Merrill

**RESUMO** - A soja é importante componente dos sistemas de produção nos cerrados brasileiros, cujos solos são ácidos e desprovidos de nutrientes. Após calagem e fertilização, as camadas inferiores ainda contêm alumínio, permanecem escassas em nutrientes e limitam o crescimento radicular, o que causa desbalançamento nutricional e estresse hídrico. As sementes podem ter alterada a sua composição química, com impacto negativo ao consumo humano e animal. Objetivou-se identificar a genética da acumulação de elementos químicos nas sementes de soja, pela análise de um cruzamento dialélico entre nove variedades, repetido em dois ambientes: alto-Al e baixo-Al. A relação entre covariância ( $W$ ) e variância ( $V$ ) mostrou que as diferenças referentes a Ca, Mg, Al e Mn são explicadas pelo modelo aditivo-dominância. Nos elementos, P, K, Fe, Zn, Cu, Mo, Na e Ti, os efeitos genéticos mostraram-se mais complexos. O nível dos elementos foi afetado pela presença do alumínio e pH, sem, contudo, comprometer a qualidade da semente. Sugerem-se estudos adicionais para elucidar a genética da acumulação de elementos químicos na semente de soja.

**Termos para indexação:** solos ácidos, alumínio, toxidez, nutriente, variedade, cruzamento dialélico.

## INTRODUCTION

The settlement of the Brazilian Cerrados (a savannah-like environment) has intensified in recent years, evolving from low input production into

modern agricultural systems, where the soybean plays a major role. Because of their poor and acidic soils, liming and fertilization are necessary for successful cultivation. However, the ploughed layer benefits mostly from the amendments and only a reduced amount of nutrients will leach down into the subsoil. Thus, cultivated plants fully adapted to these environments should possess tolerance to toxic aluminium and be efficient in nutrient utilization,

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mainly calcium, which limits root elongation (Spehar, 1989). The conjugation of these two characters allows deep rooting and tolerance to dry spells that may depress grain yield.

A major concern in Al-tolerant varieties is how the seed composition is affected. Will Al accumulate in seeds of intolerant varieties at high levels? Will its interaction with calcium and magnesium (Hecht-Buchholz & Schuster, 1987; Foy et al., 1992; Spehar, 1994c) depress these elements in seeds of Al-stressed plants? Moreover, what is the genetic mechanism that regulates accumulation of mineral elements and their relationship in the seeds?

It has been reported that in maize (Gorsline et al., 1968), in sorghum (Gorz et al., 1987) and in soybeans (Spehar, 1989), the differential absorption of nutrients in the plant is quantitatively inherited. However, little has been reported about genetic differences in seed accumulation (Spehar, 1994d).

The introduction of mathematical models into classic Mendelian genetics, has contributed to the understanding of quantitative genetic patterns and the enhancement of cultivated plants by the use of appropriate breeding schemes. The diallel set of crosses has been widely used by breeders to identify the genetics of more complex traits and at the same time in selecting new varieties (Gibori et al., 1978; Kao & McVetty, 1986). To be successfully employed, this scheme shall enhance expectancy of genetic gain relative to that of empirical classic breeding (Hanson, 1987).

The theory of diallels was developed by Jinks & Hayman (1953) and expanded by Dickinson & Jinks (1956). This procedure allows genetic analysis to be carried out and provides tests of adequacy of the genetical model (Hayman, 1954). The estimates of genetic parameters enable the breeders to guide themselves in the selection for a given trait. Predictability of its expression implies that random assortment of the genes is present, where diallel analysis allows the calculation of gene effects, whether dominance or additive. The inference of the mode of gene action is highly dependent on how far apart are the genotypes being used in the diallel cross, and how large is their number. If a small number is used, the genetic estimates are related only to the parents and crosses and not to a larger population

(Mather & Jinks, 1982). Of practical importance in breeding programmes the diallel scheme allows the identification of the lines or varieties in the crosses that contribute most to desirable traits.

There are only few examples in the literature of diallel crossing on genetic studies for mineral nutrition or toxic elements in plants. Boye-Goni & Marcarian (1985) utilized a half diallel cross of sorghum involving three varieties susceptible to Al and three tolerant varieties for genetic studies. Analysis of  $F_1$ 's indicated that general combining ability was predominant, suggesting that inheritance to Al is controlled largely by additive effects and genotypes had different contribution to Al tolerance. In a diallel study of differential accumulation of Ca, Mg and K in maize, Gorsline et al. (1968) confirmed that gene effects were mainly additive and no relation amongst these elements was found. In another experiment with hybrid forage sorghum, diallel analysis indicated that general combining ability (additive) effects exceeded specific combining ability (dominance) for mineral composition of N, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, and Zn (Gorz et al., 1987).

Because Al tolerance and efficiency of nutrient utilization are important characters, a diallel cross experiment was planned as part of the breeding programme to adapt soybeans to production systems in the tropics. It included all possible hybrids among nine Savannah conditions selected soybean varieties, tested in a high and in a low-Al environments, respectively.

The main objectives of this experiment were: to assess the genetics of mineral element accumulation by the seeds of soybeans grown in tropical acid soils, and to establish the relationship among mineral elements in the seeds of soybeans.

## MATERIALS AND METHODS

The assessment of differential response of soybean varieties to mineral element uptake in field experiments was the first step to plan hybridization for genetic studies. The following varieties were used in a diallel cross scheme: 'IAC-2', 'IAC-5', 'UFV-1', 'Vx5-281.5', 'IAC-8', 'IAC-7', 'IAC-9', 'Biloxi' and 'Cristalina'. These varieties were chosen to represent a range of variability for Al-tolerance and, with the exception of 'Biloxi', were

adapted to Central Brazil, where virgin soils need to be improved with lime and fertilizer before they can be economically cultivated (Spehar, 1994a).

A description of their genealogy is presented by Spehar (1989).

It is interesting to add that 'Biloxi', which is reported in the literature (Foy et al., 1969; Spehar, 1994b) to be Al-tolerant, has not contributed to the gene pool of the Brazilian soybean varieties.

All possible combinations of hybrids, including reciprocals, were produced in a glasshouse, at the Centro de Pesquisa Agropecuária dos Cerrados (CPAC-EMBRAPA), Planaltina, Brazil. These parent varieties were classified in distinct maturity groups and to allow coincidence of flowering, several weekly sowing batches were used, beginning with the latest varieties of this group, namely, 'IAC-7', 'IAC-9' and 'Cristalina'. Well fertilized mixture of soil and compost were used in two-liter pots in which five seeds were sown. These seeds had been obtained by selfing single plants of the parent varieties and advancing their progeny through single-seed-descent, a modified pedigree breeding method (Brim, 1966). The selfed lines exhibited the main morphological characteristics by which the original varieties were described. After emergence, the three most vigorous seedlings per pot were maintained to be used in the crosses. The technique for hybridization was adapted from Fehr (1980).

The following genetic markers were used in the  $F_1$  generation to distinguish between crossings and selfings: colour of the flower and hypocotyl (purple dominant to white), pubescence colour (brown dominant to grey), pod colour (brown dominant to tan), growth habit (indeterminant dominant to determinant), maturity (long cycle dominant to short cycle), seed colour (yellow dominant to brown) and hilum colour (black dominant to brown).

Given the natural difficulty in handling the minute soybean flowers, and the number of crosses which were required, the few  $F_1$  seeds were advanced to  $F_2$  in the glasshouse and with supplemental light, to generate a larger bulk of seeds for the diallel experiment in the field.

The experiment was conducted in two environments in the field. The two areas in which the experiment was repeated were fertilized in the following manner: 1) 500 kg/ha dolomitic limestone (100 %  $\text{CaCO}_3$  equivalent), 150 kg/ha P, 75 kg/ha K and 40 kg/ha slow release micronutrients source, FTEBR-12; 2) 4,000 kg/ha dolomitic limestone (100%  $\text{CaCO}_3$  equivalent) and the other sources of nutrients in the same amounts as in the first case. They were named high and low-Al areas. The chemical analyses of soil samples collected after the

experiment indicated pH ( $\text{H}_2\text{O}$  1:1) 5.1 and 5.7; Al 0.97 and 0.10 cmol/kg; Ca+Mg 1.65 and 3.56 cmol/kg; P 3.1 and 3.4 mg/kg; K 51 and 58 mg/kg, respectively.

To allow testing of all hybrids, the hill plot scheme was used. Hill plots were shown to be an efficient alternative to row plots for selection of soybean varieties in the Brazilian Cerrados (savannahs) when there is shortage of seeds (Spehar, 1989). Ten  $F_2$  plants per hill were grown and constituted one plot. Three replications were used. The hills were 0.70 m apart in one direction and 1.00 m in the other, which was shown to be sufficient to avoid interplot competition. The details of soil amendments and their incorporation into the soil in the two areas of the experiment were described by Spehar (1994c).

Individual plants were harvested at maturity separately from each hill. Ten seeds of each of 30  $F_2$  plants were used to make a composite sample to examine the mineral composition of the soybean crosses. The reciprocal crosses were not included in this sampling. The seeds, oven dried to stabilize for moisture, were milled to a fine powder. A sample of 0.5 g, weighed to the nearest mg, was taken for mineral analysis. The soya flour was placed in an acid washed silica crucible and dry ashed in a muffle furnace at 450°C for 12 hours. This turned it into whitish ash without volatilization. After cooling, the samples were dissolved in 5 ml 6M HCl (v:v) and placed in a steam bath until dryness. The process was repeated. After they were dry, 5 ml of HCl (1:1) was added to the crucibles to dissolve the soluble residue. Samples were transferred quantitatively with washings to a 50 ml volumetric flask. After settling of the residue, aliquots were pipetted for analysis in the ICPEs.

The diallel analysis for seed mineral composition was performed according to the method described by Hyman (1954) for a half-diallel. The statistical method permitted the calculations of  $V_r$  and  $W_r$ , which are the variance of an array and the covariance of the offspring in an array with the non-recurrent parents, respectively. There were nine arrays and each array consisted of nine families, i.e., the parent and its eight crosses with the other parents. If the assumptions of the model for diallel analysis are satisfied, i.e., diploid segregation, no reciprocal differences, independent action of non-allelic genes, no multiple allelism, homozygous parents, and independent distribution of genes, the linear regression of  $W_r$  on  $V_r$  has a slope which is not significantly different from 1. A t-test was used on regression of  $W_r - V_r$  values to verify if the additive-dominance model explained the genetic pattern of segregation for the observed characters.

## RESULTS AND DISCUSSION

The mean values over arrays for seed chemical analysis are presented in Table 1. The analysis of chemical composition has shown the range for aluminium in the seeds is not high in both high and low-Al environments, even though Al levels were up to three fold higher in the leaves of stressed plants (Spehar, 1993). This confirms previous work where excess leaf Al was not transferred into the seeds (Spehar, 1994d).

There were minor differences among arrays means when compared to corresponding high differences in the leaves (Spehar, 1989). The same trend was observed for Fe, positively associated with Al in the leaves (Spehar, 1993).

The levels of P and K in the seeds varied in the range of 0.045 to 0.023 and 0.098 to 0.037% for the lowest to the highest array mean in the high and in the low-Al environment, respectively. The highest differences for Ca were observed when the common parent was the variety 'UFV-1', already shown to

TABLE 1. Array means for mineral elements in soybean seeds of the 9 x 9 diallel cross from high (H) and low-Al (L) experiments.

Element	Al	Array								
		IAC-9	IAC-2	UFV-1	IAC-5	IAC-8	Vx5-281	IAC-7	Biloxi	Cristalina
Phosphorus (g/100g)	H	0.622	0.621	0.624	0.616	0.607	0.647	0.628	0.625	0.602
	L	0.519	0.515	0.538	0.522	0.522	0.534	0.519	0.535	0.522
Potassium (g/100g)	H	1.917	1.882	1.940	1.896	1.842	1.871	1.855	1.850	1.859
	L	1.740	1.830	1.861	1.850	1.724	1.748	1.731	1.786	1.785
Calcium (g/100g)	H	0.216	0.238	0.235	0.230	0.216	0.240	0.220	0.210	0.219
	L	0.229	0.258	0.260	0.244	0.229	0.256	0.229	0.239	0.236
Magnesium (g/100g)	H	0.249	0.251	0.260	0.260	0.255	0.262	0.262	0.256	0.248
	L	0.246	0.253	0.258	0.254	0.254	0.261	0.255	0.253	0.248
Iron (mg/kg)	H	98.2	108.1	98.2	113.0	116.9	116.4	111.8	110.1	108.1
	L	85.9	88.3	91.8	90.5	95.9	91.8	89.1	90.3	90.0
Aluminium (mg/kg)	H	9.4	7.1	8.5	8.7	9.2	9.5	8.3	8.7	8.8
	L	4.4	5.8	4.8	5.2	4.0	4.7	4.4	3.7	3.9
Manganese (mg/kg)	H	44.1	46.5	46.2	45.6	42.7	47.0	44.1	42.3	42.8
	L	24.8	26.7	26.8	25.5	26.1	26.4	25.0	25.8	25.3
Zinc (mg/kg)	H	55.9	58.2	58.3	58.8	56.0	60.3	59.3	58.1	55.8
	L	41.0	44.1	44.8	43.4	40.7	44.8	42.1	44.0	42.2
Copper (mg/kg)	H	11.4	10.9	10.4	11.0	10.5	11.4	10.7	11.1	10.6
	L	9.4	8.5	8.5	8.6	8.1	8.0	8.4	8.4	8.3
Molybdenum (mg/kg)	H	1.56	1.14	1.04	1.35	1.53	1.50	1.26	1.25	1.23
	L	1.37	1.24	1.02	1.13	1.18	1.21	1.00	1.00	0.98
Sodium (mg/kg)	H	29.8	29.9	29.0	25.1	24.6	26.0	28.5	25.9	26.9
	L	22.9	23.8	22.4	21.1	24.4	22.3	22.5	24.1	23.6
Titanium (mg/kg)	H	1.46	1.12	1.20	1.25	1.28	1.60	1.27	1.28	1.27
	L	1.22	1.22	1.06	1.00	0.90	1.15	1.07	1.13	1.14

be Al intolerant and Ca inefficient (Spehar, 1994c). The possible cause for this may be the concentration of root growth in Ca-rich areas of the soil.

The levels of Ca and Mg were associated in the leaves (Spehar, 1993) but had a different performance in seeds, i.e., Ca tended to be higher in the low-Al environment while Mg did not show difference as measured by the mean over arrays, although the array means for crosses with the intolerant varieties 'UFV-1', and 'Vx5-281.5' were greater than the arrays for tolerant 'Biloxi' and 'IAC-9', confirming previous report for these varieties (Spehar, 1994d). It seems that there is a relationship between Ca, Mg and Al in seeds, although not as clear as to that detected in other plant parts (Hecht-Buchholz & Schuster, 1987; Foy et al., 1992; Spehar, 1993).

Mn, Zn, Cu, Mo, Na and Ti did not show a trend among the arrays, which indicates lack of variability for these elements. It is possible that some discrepant values in the analysis for these elements contributed to increase the error.

The results of the regression analysis for the relationship of covariance ( $W_r$ ) on variance ( $V_r$ ) are presented in Table 2. A proof of whether the model was valid carried out with  $t$  tests for the regression of slope  $b = 1$ . The  $t$  values were highly significant for most of the elements and only for Ca, Mg, Al and Mn were non-significant. This is an indication that the additive-dominance model explains the genetic differences among the genotypes for these elements. The apparent discrepancy between the high and the low-Al was probably as a result of reduced root growth, causing differential nutrient uptake in the two areas. Due to difficulty in handling large numbers of samples for seed analysis, only one composite over replications was available and this limited the amount of statistical analysis that could be performed. However, comparisons with results for diallel analysis on leaf composition (Spehar, 1989), suggest that additive effects are greater than dominance effects for the accumulation of mineral elements in soybean seeds, even though there were heterotic combinations that may have been the cause for lack of fit of the model. The genetic variability for manganese is less expressed due reduced magnitude of varietal response. This can be a

**TABLE 2. Regression of covariance ( $W_r$ ) on Variance ( $V_r$ ) for mineral composition of soybean seeds from high (H) and low-Al (L) experiments.**

Element	Al	Regression equation	Test for $b=1$	
			t	p
Phosphorus	H	$0.0001 + 0.113V_r$	4.39	0.004
	L	$-0.0002 + 0.267V_r$	3.35	0.009
Potassium	H	$0.0015 + 0.085V_r$	4.33	0.004
	L	$0.0032 + 0.101V_r$	6.46	<0.001
Calcium	H	$-0.0001 + 0.726V_r$	1.06	0.296
	L	$-0.0003 + 1.080V_r$	0.27	>0.500
Magnesium	H	$0.0001 - 0.226V_r$	5.22	0.002
	L	$0.0001 + 0.470V_r$	1.78	0.120
Iron	H	$125.78 - 0.012V_r$	5.62	0.001
	L	$25.81 + 0.016V_r$	7.40	<0.001
Aluminium	H	$2.577 - 0.096V_r$	5.29	0.001
	L	$-5.853 + 0.493V_r$	2.02	0.090
Manganese	H	$0.642 + 0.480V_r$	2.97	0.023
	L	$-4.700 + 0.924V_r$	0.47	>0.500
Zinc	H	$-0.043 + 0.5166V_r$	7.20	<0.001
	L	$1.699 + 0.1104V_r$	11.21	<0.001
Copper	H	$-0.250 + 0.3364V_r$	2.59	0.035
	L	$-0.011 + 0.1402V_r$	5.99	<0.001
Molybdenum	H	$0.173 + 0.3389V_r$	4.20	0.004
	L	$-0.009 - 0.0104V_r$	6.07	<0.001
Sodium	H	$-11.129 + 0.0064V_r$	8.92	<0.001
	L	$-6.713 + 0.0791V_r$	6.70	<0.001
Titanium	H	$0.132 - 0.1447V_r$	6.34	<0.001
	L	$0.030 - 0.0681V_r$	8.89	<0.001

consequence of its pH-dependent chemical peculiarity (Ritchey et al., 1982). Plant pH regulates its internal mobility even when leaf-Mn is sufficient, thus reducing differences in seeds.

The present diallel analysis, although not conclusive, made it evident that the genetics of mineral element accumulation in the seeds of soybeans is not fully associated to the genetics of mineral elements in the leaves, although its expression is dependent on mineral plant-stress. It may be interesting to emphasize that, although there were genetic differences in the germplasm as detected by

the arrays, the lower levels of Al in the seeds than in the leaves is advantageous for humans or livestock. It seems that most of the Al x nutrients interactions take place in other plant parts, especially the root. Breeding for Al tolerance in the soybeans should not bring negative consequence for the chemical composition of the seeds, with no effect on food quality.

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