Maize Genetic Resources with Contrasting Phosphorus Efficiency

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In Brazil, over the past two and a half decades, nearly 15 million hectares of the vast acid savannas or "cerrado" have been brought into crop production. The Brazilian cerrado comprises an area of over 200 million hectares of which 112 million are considered arable. Currently about 30% of the Brazilian maize crop is produced in the cerrado occupying an area close to 3.5 million hectares. This has been possible due to the effort of many public and private research programs working on crop production and breeding for acid soils. Among these institutions, the Embrapa Maize and Sorghum Research Center started a maize breeding program for adaptation to acid soils in the late nineteen seventies. A research approach of simultaneously screening and testing genotypes in both acid soils and non-acid fertile soils, coupled with nutrient solution screening with an Al gradient allowed for the identification of maize germplasm sources adapted to acid soil and with good levels of Al tolerance. The breeding approach started with massive screening of tropical germplasm in acid soils with different levels of aluminum saturation (Bahia Filho et al., 1997). Using these results, basic populations (composite and synthetics) have been synthesized and improved (Lopes et al., 1987). Inbred lines have been extracted from these populations and contrasting genotypes were used to calibrate screening techniques (nutrient solution and soil) to assess aluminum

tolerance (Magnavaca, 1982). These populations were submitted to recurrent selection and their combining ability and heterotic groups were determined (Lopes et al., 1987; Gama et al., 1986; Eleutério et al., 1988). A large number of inbred lines were developed from this germplasm and superior testers have been developed (Magnavaca et al., 1990). Hybrids with good adaptation to acid and fertile soils have been developed and released for farmer use since 1986 (Magnavaca et al., 1988).

In the first phase of the maize breeding program for acid soil adaptation we have concentrated our efforts in studying aluminum tolerance related to adaptation to acid soil environments (Magnavaca et al., 1987c). A second step in these studies has been towards the determination of genotypes with better adaptation to phosphorus stress in the soil (Alves, 1994; Alves et al., 1988; Parentoni et. al., 1999). This shift was based on research data accumulated over the last two decades from plant nutrition scientists working on acid soils of the tropics. A summary of the key points of these studies as described by Novais and Smith, 1999 are: a) the cerrado soils have low water holding capacity and the rain distribution in this area is irregular leading to a scenario of repeated wetting and drying of the soil profile during the crop growing season; b) the oxisols in the area have high phosphorus fixing capacity, and the soil competes with the plant for the low amount of available P; c) even with a small reduction in water content (much before reaching the wilting point), the availability of P from the soil is reduced and this phenomenon also worsens as the clay content in the soil increases; d) the P stress in the plant affects its internal water status and also reduces the absorption of nitrogen. These facts point in the direction that

P nutrition controls a cascade of effects that determine a large proportion of the plant adaptation to acid savannas environment (Novais and Smith, 1999).

Based on the rational described above, for the past six years, the maize breeding program for acid soil adaptation at Embrapa has been focusing on identifying maize genotypes contrasting for P efficiency (Parentoni et al., 1999). Up to now, there is no available lab screening technique for determining P efficiency en maize, like, for example, the nutrient solution system used to screening for aluminum tolerance. In this case, a suitable approach is to develop soil based screening techniques. A important point to be considered in developing a soil based screening method for P efficiency for maize, is the fact that, in contrast with what has been observed for soybean, split root experiments in soil compartments with and without phosphorus, have shown that maize needs to have a good P supply along all root system in order to have a well P nutrition in the plant (Novais and Smith, 1999). This would be one of the reasons that soybean is the crop used to be planted in recently open savana areas and maize will only became part of the rotation system 3 to 5 years later, when a good P distribution has been achieved in the area. Due to that fact, a soil screening methodology for P use efficiency in maize has to be done in a previously prepared area with adequate levels of P in all the root zone profile. Having these points in mind, a field screening methodology for P efficiency was developed using a uniform Red Oxisol clay texture soil. The first step was to determine an adequate P level for pursuing the screening. The idea was to look for P levels in the soil that would allow the identification of maize genotypes better able to use the phosphorus applied in the soil as fertilizer. Originally, the natural P level in this area was below 1 ppm. Previous experiments have determined that the P critical level for maize grown in this soil was close to 10 ppm. A experimental area was then divided into two sub areas and the P level of one of the sub-areas was corrected to the critical level (10 ppm) and the other sub area was corrected to half of the soil P critical level or 5 ppm of P. In the summer of 1994 a group of 100 maize single cross hybrids obtained from inbred lines previously selected for acid soil adaptation were evaluated in both areas (Parentoni et al., 1996). Superior genotypes would be the ones with the smallest reduction in yield across P levels and the highest yield under the low P level. A mean reduction in yield of 16.7% across P levels was observed for the 100 genotypes evaluated. The range of yield reduction across P levels was from less than 1% up to 40%. This mean differences in yield across P levels was considered small and we start to search for contrasting P levels in the soil that would lead to a mean yield reduction across P levels between 30 and 40%. These results have been obtained in more recent field experiments using P levels in the two areas of 2 and 15 ppm. We have found that this yield reduction (30 to 40% mean yield reduction across P levels) was adequate to discriminate between P efficient and P inefficient maize genotypes. Many of the single crosses evaluated in the first screening of 100 single crosses conducted in 1994 had parental inbred lines in common. Based on the yield of the single crosses in both P levels, it was possible to determined a group of inbred lines that participated as parents in single crosses classifyed as phosphorus efficient and P responsive and it was also identified another group of inbred lines with higher frequency as parents in crosses with low phosphorus efficiency. This information was used to select inbred lines to be used as parents in diallels to be evaluated under P stress and non-stress condition.

Since 1994 we have evaluated a total of 589 maize genotypes (mainly hybrids and a small group of OPVs) for P efficiency. The selection criteria has been yield in environments with and without P stress. A selection index has been proposed, based on two components. The first component is the product of the yield values for genotype "i" under P stress (Ai) and without P stress (B i), divided by the product of the mean of all genotypes evaluated in this trial in both environments ($\bar{A}$ x $\bar{B}$). These can be considered a "breeding component", since it will pick up the genotypes with higher yields in both environments. Although, in this case, genotypes having medium yield under P stress condition but been highly responsible to P could also be picked up using this selection index. To avoid this problem, a second component was introduced in the index. This component would be the yield of genotype "i" under P stress, divided by the mean of all genotypes in the P stress environment ($\bar{A}$). The selection index would then be: [(Ai x Bi) / ($\bar{A}$ x $\bar{B}$)] x [Ai / $\bar{A}$]. This index was then applied to the 589 hybrids evaluated in 9 different trials and the following properties were observed: a) in each hybrid trial the index in general ranges from 0 to a maximum value of 5; b) hybrids with index values superior to 2 can be considered superior. We try to use this index to get a rank of all genotypes evaluated for P efficiency in the past 6 years. Among the selected efficient genotypes we try to selected the ones with highest value of (Ai/Bi), since this index has been commonly used for physiology studies. In the same way, in selecting for P inefficient genotypes we picked up the ones with smaller index value and low values of (Ai/Bi). A summary of the grain yield values for the more contrasting P genotypes (efficient and inefficient) identifyed among the 589 ones evaluated under two P levels in the soil is

Table 1. Standard hybrids for P efficiency and P inefficiency identified based on yield evaluation (kg of grain/ha) in a Red Oxissol with 2 and 15 ppm of P. A selection index (see text above) was used to pick up the contrasting hybrids among 589 genotypes evaluated from 95/96 to 99/2000. Embrapa Maize and Sorghum , Sete Lagoas, MG. Brazil.

Trial YearTreat. P Efficient Standard Hybrid	Hybrid	A Yield -2 ppm	B Yield 15 ppm P	A/B	Stress Index
HSDI-9-9697-05	11X36	6391*	6945	0,92	3,71
HS9899-03	26 X 161-1	7770	10890	0,71	3,44
HS9697-77	161x723726-45	7235	7061	1,02	2,90
HS9798-53	BRS 3060	8107	8898	0,91	2,74
HS9899-77	228-3xL3	6882	10010	0,69	2,48
HS9495-54	20X723	4800	4708	1,01	2,48
HS9899-30	26XL3	6771	9679	0,70	2,32
HS9899-72	228-3x36	7258	7779	0,94	2,19
HS9899-65	36 x L 3	6882	8184	0,84	2,03
P Inefficient Standard Hybrids					
HS9697-25	5046x53	2752	4155	0,66	0,25
HS9899-33	2841X5046	3219	6489	0,50	0,35
HS9899-29	26X1113-01	3207	6930	0,46	0,37
HS9495-97	BR 206	2440	4116	0,59	0,38
HSDI-9-9697-17	16X22	2320	5417	0,43	0,38
HSDI-9-9697-18	16X36	2620	4517	0,58	0,41
HSDI-9-9697-20	16X723	2722	4353	0,63	0,42
HS9495-13	20X22	2394	4865	0,49	0,43
HS9495-04	16X22	2425	5202	0,47	0,47
HS9697-60	26X22	3501	5337	0,66	0,51
HS9899-39	2891x5046	3441	8359	0,41	0,52

**Kernel weight was obtained by multiplying ear weight by 0.75*

shown on Table 1. In the past years, close to 80 maize inbred lines were also evaluated "per se" for P use efficiency. Based on our preliminary results on inbred line "per se" evaluation we were able to identify two contrasting groups for P efficiency. Within each group we also identified inbred lines with high or low "per se" yield potential. The main representatives of each of these groups are: a) P efficient and high yielding inbred lines (L 3, L 228-3, L 28.41, L 36); b) P efficient and medium to low yielding inbred lines (L 161-1, L 11); c) P inefficient and high yielding inbred lines (L 53, L 726); d) P inefficient and medium to low yielding inbred lines (L 13, L Cat. Col. 96/ 71 and L 16).

The ability of an inbred line to produce hybrids with higher or lower levels of P efficiency was also investigated. Three diallel crosses involving 8, 9 and 13 inbred lines, comprising respectively 28, 36 and 78 single cross hybrids were evaluated under P stress and P non stress conditions. Some relevant points observed in these studies were: a) the ratio between additive and non additive gene effects (Φg / Φs) for yield were higher under P stress conditions, than under non P stress conditions, indicating a relative higher importance of additive gene effects under P stress; b) the interactions of general combining ability (GCA) x P levels were significant for all three diallels, while the interaction of specific combining ability (SCA) x P levels have not been significant.

Changes in General Combining Ability (GCA) values from the non P stress environment (15 ppm of P) to the P stress environment (2 ppm of P) were used to identify inbreds contrasting for its capability to produce hybrids with higher or lower degree of adaptation to P stress environments. Inbreds like L3, L161-1, L11, L36 and L723 will produce hybrids more adapted to P stress environments. On the other hand, inbreds like L 228-3, L22, L28.91, L16, L 26 and L 724 will produce hybrids more adapted to soils with no P stress (Tables 2, 3 and 4). The latest group of 13 inbred lines evaluated in a diallel scheme in the summer of 1998/99 confirmed the progress being achieved in maize adaptation to P stress environments

Table 2. Ear weight (kg/ha) effects of general combining ability (GCA) for a diallel between 8 maize inbred lines. The diallel was evaluated in a Red Oxisol under two levels of phosphorus: 5 ppm (P stress) and 10 ppm in Sete Lagoas, MG, Brazil.

Inbred	Origin	CGA 5 ppm P (kg/ha)	CGA 10 ppm P (kg/ha)
L 11	Pool 25	1049	953
L 13	Pool 25	-416	-383
L 36	Recycling CNPMS	236	952
L 64	Pool 22	99	-691
L 723	Population 27	943	453
L 726	Suwan	83	338
L 1143	Suwan	-1026	-1159
L 1167	Suwan	- 968	-463

in our breeding program. New inbreds like L3 and L 161-1 demonstrated tremendous potential to produce hybrids with superior performance in P stress environments (Table 4).

The last diallel cross evaluated under P stress and non stress conditions (13 parents inbred lines and 78 F1 crosses) was also used to evaluate P content in kernels and stove. Under P stress condition, an almost linear relationship (R^2 = 0.73) was observed between plant absorbed P (mg/plant) and grain yield (g/plant). These fact was not observed under P non stress condition, indicating that other factors than P nutrition would be limiting the plant yield in this environment or another hypothesis would be that P luxury consumption**

Table 3. Ear weight (kg/ha) effects of general combining ability (GCA) for a diallel between 9 maize inbred lines. The diallel was evaluated in a Red Oxisol under two levels of phosphorus: 2 ppm (P stress) and 15 ppm in the summer of 1996/97 in Sete Lagoas, MG, Brazil.

Inbred	Origin	CGA 2 ppm P kg/ha	CGA 15 ppm P kg/ha
L 11	Pool 25	747	1068
L 13	Pool 25	-538	-662
L 16	Tuxpeno	-1155	-345
L 20	Tuxpeno	4	-212
L 22	Tuxpeno	-86	281
L 36	Recycling CNPMS	403	-157
L 64	Pool 22	-92	-149
L 723	Population 27	653	-31
L 724	Tuxpeno	64	207

could be occurring in the high P environment.

From the large groups of single cross hybrids evaluated during the past 6 years, it was possible to identify highly contrasting genotypes for phosphorus efficiency. It should be noticed that, if inbred lines will be used for basic studies (mechanisms or molecular biology studies), the choice of contrasting inbred lines for P use efficiency has to be based on inbred line "per se" evaluation under soil P stress and non stress conditions. Otherwise, if the goal is to develop superior hybrids for P stress and non stress conditions, the combining ability of the lines in these two different environments would

Table 4. Ear weight (kg/ha) effects of general combining ability (GCA) for a diallel between 13 maize inbred lines. The diallel was evaluated in a Red Oxisol under two levels of phosphorus: 2 ppm (P stress) and 15 ppm in the summer of 1998/99 in Sete Lagoas, MG, Brazil.

Inbred	Origin	CGA 2 ppm P kg/ha	CGA 15 ppm P kg/ha
L 26	Tuxpeño	56.1	437.8
L28.41	Tuxpeño	-107.8	68.0
L 28.91	Tuxpeño	-1054.3	-117.8
L 161-1	Tuxpeño	857.3	1510.7
L 1096	Tuxpeño	-264.8	-1047.8
L 228-3	Tuxpeño	-162.7	1164.2
L 36	Recycling CNPMS	463.4	-531.8
L 723726-45	Population 27	139.6	-420.3
L 1154	Suwan	194.9	-526.6
L 5046	CMS 50	-431.6	-756.5
L 1113-01	Pool 25	-342.6	-40.8
L 3	Recycling CNPMS	1132.2	1121.2
L 1057-68	Pool 25	-479.6	-860.3

be the main selection criteria to pick-up the best parents for a hybrid breeding program.

The contrasting maize genotypes for P use efficiency identified in our breeding program have been used by plant nutrition researchers to study mechanisms involved in P efficiency. We expect that these studies will allow the development of more refined screening techniques. Contrasting inbred lines for P efficiency have being crossed and segregating populations are being produced from these crosses. These materials will be used to map P efficient genes. We plan to develop a set of recombinant inbred lines (RILs) from one of these crosses. We anticipate that this will be an invaluable resource to study phosphorus efficiency in maize.

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