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"Fitting maize into cropping systems on acid soils of the tropics"

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Summary:

During the four years of this project, our knowledge about physiological and molecular plant processes induced by aluminum (Al) stress and phosphorus (P) stress has significantly increased. Breeding efforts at Embrapa Maize and Sorghum have identified maize genotypes contrasting in both tolerance to Al stress and P acquisition/utilization efficiency under P stress. Tolerance to Al toxicity has been present in several new maize cultivar releases. The recent release BRS 3060, a triplecross maize hybrid, is both tolerant to Al toxicity and more efficient in P acquisition in P stress conditions. BRS 3060 is one of the best benchmark standards for P acquisition efficiency in P stress soils. These genetic standards have been distributed to project partners where they have been used to conduct whole plant and molecular physiology-based research to determine mechanisms for tolerance to Al toxicity and differences in P acquisition processes. Identification of these mechanisms is important to help direct crop improvement programs, particularly those based on plant biotechnology. Recombinant Inbred Lines (RILs) derived from a cross of contrasting maize lines for tolerance to Al toxicity have been used to identify QTLs associated with tolerance to Al toxicity. Six QTLs have been identified that explain 60% of variation of relative seminal root growth (RSRG) in nutrient solution with Al. Improved cultivars have been developed using technology and genetic resources developed at Embrapa Maize and Sorghum and released to private sector seed producers, and will continue to be developed and released independent of this project, but building on the technology generated from this project.

1. Background:

The soils of the tropical savannas, an area of approximately 850 million hectares, are some of the oldest in the world. These soils have generally been degraded naturally over the past millions of years. Low fertility, low pH, low P availability, high P sorption capacity (fixation) and toxic levels of aluminum commonly characterize them. These naturally degraded infertile acid soils have been one of the principal factors limiting development and food production in many countries throughout the tropics, an area that contains 58 percent of the world's land area suitable for agriculture production as well as 73 percent of the world's population. There is also a vast area of low fertility non-acid soils in the tropics that are low in P due to natural or man made soil fertility degradation.

The opening and development of the expansive tropical acid savanna or cerrado of Central Brazil was enhanced with the transfer of the Brazilian capital from Rio de Janeiro to Brasilia in 1960. Growth and development of Brasilia, located on the central plateau in the center of the cerrado ecosystem, also had a positive influence on agricultural development in this seemingly hostile naturally degraded environment for agriculture production. Over the past three decades, more than 16 million hectares of the approximately 215 million hectares of the acid savanna or cerrado of Central Brazil have been brought into sustainable crop production. Currently over 30% of Brazil's rice, maize, and soybean crops, 20% of its coffee, and 15% of its edible beans are produced in the cerrado. For example, the area planted with maize in the cerrado increased from 1.6 million hectares in 1970 to over 3.5 million hectares today while the average productivity has risen from less than 1.4 t/ha to nearly 4.0 t/ha. Also, over one million hectares of the cerrado will be planted to sorghum this year. Total grain production (rice, maize, beans, soybeans, sorghum and wheat) in the cerrado has increased from 5.6 million tons in 1970 to over 30 million tons in 2000, comprising approximately one-third of the total grain production in Brazil. An additional 35 million hectares of improved pastures have been developed in the cerrado carrying 53 million head of cattle that produce 40% of Brazil's meat and 12% of its milk.

This successful transformation of the Brazilian cerrado into productive agricultural land can be attributed to the results of interdisciplinary and multidisciplinary crop production and improvement research programs. This transformation evolved in three phases over the past 25 years. First, technology and programs were developed to reduce soil acidity and improve soil fertility with applications of lime and phosphorus. This in itself was not sufficient to develop sustainable production systems due to the impossibility of correcting subsoil acidity and the high P fixing capacity of the soil. Coupled with the technology of ameliorating the soil constraints, plant-breeding teams developed modern high yielding cultivars with tolerance to aluminum toxicity and improved P acquisition efficiency. These cultivars develop roots with the ability to penetrate into the acid subsoil with high levels of aluminum saturation, which improved both water uptake and nutrient use efficiency. Apparently some of these cultivars also have the ability to modify the rhizosphere environment and increase the efficiency of P acquisition. In recent years these technologies have been associated with the development of no-till or direct planting production systems (third phase) reducing environmental degradation and maintaining higher levels of soil organic matter. Over the past twenty years, Brazilian scientists at Embrapa and other research institutions, including the partners of this project, have made progress in understanding mechanisms of abiotic stress tolerance and in developing genetic resources with improved and more stable yield in tropical soils. These developments are expected to be of similar magnitude as those developed for biotic stress factors during the past century.

Several mechanisms have been proposed for ameliorating these abiotic stresses, such as the exudation of organic acids to detoxify toxic Al and release sorbed P into the rhizosphere, the production and release of phosphatases to utilize organic phosphate more efficiently, the development of a larger and more profuse root system to enhance nutrient acquisition, and an increased efficiency of phosphate transporters. Recent research has shown that the presence and exudation of citrate and its complex with Al is a principal mechanism for Al tolerance in maize. The presence and exudation of malate may also be a factor in tolerance to Al toxicity. Understanding the mechanisms involved in providing tolerance to Al toxicity and in increasing the efficiency of P acquisition in soils with low P and high P fixing capacity is expected to increase the efficiency of developing new and improved high yielding cultivars with both tolerance to Al toxicity and more efficient P acquisition. Progress in developing new cultivars using traditional plant improvement techniques has been slow and laborious. The identification and development of physiological and molecular determinants for tolerance to Al toxicity, more efficient P acquisition, global regulation of gene expression, and the utilization of genomics information is expected to enhance the efficiency of selection of superior genotypes.

Objectives:

The research objectives of this project by Embrapa were: 1) to identify and develop genetic resources for Al tolerance and P efficiency in maize, 2) to exchange maize germplasm standards with project collaborators, 3) to develop improved germplasm and high stable yielding maize cultivars, and 4) to understand the mechanisms that control the tolerance to Al toxicity and improved P acquisition efficiency under conditions of soil P stress in maize genotypes, facilitating the development on high yielding maize cultivars with improved efficiency in P absorption and utilization.

Activity 1 – Genetic Resources and Germplasm Standards

Using a research approach of simultaneously screening and testing genotypes in both acid soils and non-acid soils, coupled with nutrient solution screening with an Al gradient, sources of maize germplasm tolerant to Al toxicity has been identified, and high yielding cultivars have been developed. Similarly, screening and testing genotypes in soils with a P gradient, sources of maize with improved P use efficiency have been identified, and high yielding cultivars have been developed.

A refined set of genetic standards for tolerance to aluminum toxicity (Table 1.) was developed and assembled at Embrapa. This was accomplished through field screening at intermediate levels of Al toxicity (36% – 50%) and screening in nutrient solution with and without toxic levels of Al. Both open pollinated varieties and hybrids characterized at Embrapa were sent to all the collaborators of this project for field evaluation and/or use in studying mechanisms of tolerance. The on-going maize improvement program at Embrapa has used and continues to utilize this elite germplasm to develop improved high yielding cultivars for Brazilian maize producers.

A set of genetic standards for more efficient phosphorus acquisition efficiency (Table1.) was also developed and assembled at Embrapa. This was accomplished through field screening at two levels of phosphorus, less than 50% P critical level (2 – 5 ppm P) and equal to or greater than 100% P critical level (> 10 ppm P). Single, three-way and double-cross hybrids characterized at Embrapa were sent to all the collaborators of this project for field evaluation and/or use in studying mechanisms of more efficient P acquisition. The on-going maize improvement program at Embrapa utilized this elite germplasm to develop improved high yielding cultivars for Brazilian maize producers. BRS 3060, one of the best standards for P efficiency has also been released for commercial production in Brazil.

The sets of genetic standards for Al toxicity and P acquisition efficiency (Table 1) were distributed to the project partners; (Dr. Walter Horst (Germany), Prof. Juan Barcelo (Spain), Dr. Charles The (Cameroon), Dr. Henri Calba (CIRAD France), Dr. Claude Welker (CIRAD-Guadeloupe), and CORPORICA in Colombia. These standards have been employed in experiments with objectives of understanding plant-nutrient interactions and in better understanding the underlying physiological mechanisms that control both tolerance to Al toxicity and improved P acquisition efficiency.

Table 1. Maize genetic standards developed at Embrapa Maize and Sorghum for tolerance to Al toxicity and P acquisition efficiency and made available to project partners.

Phenotypic Characteristic	Type of Genetic Resource	Name
Tolerant to Al stress	Open pollinated variety	CMS 36

Tolerant to Al stress	Open pollinated variety	CMS 30
Tolerant to Al stress	Open pollinated variety	CMS 04C
Tolerant to Al stress	Open pollinated variety	CMS 14C
Tolerant to Al stress	Open pollinated variety	CMS 13
Susceptible to Al stress	Open pollinated variety	Tuxpeno Sequia
Susceptible to Al stress	Open pollinated variety	BR 106
Susceptible to Al stress	Open pollinated variety	BR 126
Susceptible to Al stress	Open pollinated variety	CMS 11
Susceptible to Al stress	Open pollinated variety	CMS 12
Tolerant to Al stress	Singlecross hybrid	1143 X 13
Tolerant to Al stress	Singlecross hybrid	1143 X 64
Tolerant to Al stress	Singlecross hybrid	13 X 64
Susceptible to Al stress	Singlecross hybrid	36 X 723
Susceptible to Al stress	Singlecross hybrid	723 X 726
Susceptible to Al stress	Singlecross hybrid	11 X 723
Susceptible to Al stress	Singlecross hybrid	11 X 36
Tolerant to Al stress	Doublecross hybrid	SHD 91102
Tolerant to Al stress	Doublecross hybrid	HD 9153
Tolerant to Al stress	Doublecross hybrid	HD 9110
Tolerant to Al stress	Doublecross hybrid	HD 9176
Tolerant to Al stress	Doublecross hybrid	BR 201 - M
Susceptible to Al stress	Doublecross hybrid	HD 91101
Susceptible to Al stress	Doublecross hybrid	HD 91107
Susceptible to Al stress	Doublecross hybrid	HD 9148
P Efficient	Singlecross hybrid	HS 36 X 22
P Efficient	Singlecross hybrid	HS 20 X 22
P Efficient	Singlecross hybrid	HS 36 X 64
P Efficient	Singlecross hybrid	HS 36 X 22
P Efficient	Singlecross hybrid	HS 20 X 723
P Inefficient	Singlecross hybrid	HS 13 X 16
P Inefficient	Singlecross hybrid	HS 11 X 723
P Inefficient	Singlecross hybrid	HS 64 X 724
P Inefficient	Singlecross hybrid	HS 13 X 1143
P Efficient	Triplecross hybrid	BRS 3060

A population of 168 recombinant inbred lines (RILs) for Al toxicity were developed by crossing two contrasting inbred lines (L53, Al susceptible and L1327, Al tolerant) for tolerance to Al toxicity. These RILs were used to identify and map quantitative trait loci (QTLs) for Al toxicity. An expanded set of RILs for tolerance to Al toxicity and a set of RILs for variation in P acquisition are currently under development.

A group of 64 singlecross and threeway hybrids was evaluated at Sete Lagoas on a red Oxisol at two levels of phosphorus (2 ppm and 15 ppm). The mean reduction in yield across P levels was 23% (2114 kg/ha). The genotypes were classified based on the ear weight under the low P level and the ratio of yield under low and high P level. The results for the Brazilian genotypes distributed among the project partners are shown on Table 2. The triplecross, BRS3060 and the singlecross, HS 20x723 showed the highest level of P efficiency and the single crosses HS 11x723, HS 13x1143 and double cross HD9148 showed low P efficiency.
The availability of there contrasting genetic resources for tolerance to Al toxicity and P efficiency, developed at Embrapa Maize and Sorghum, have been extremely useful to the partners of this project to investigate the mechanisms associated with tolerance to Al toxicity and improved P efficiency.

Table 2. Ear weight (kg/ha) for selected hybrids of a 64 hybrid yield trial evaluated at 2 P levels (2 and 15 ppm). Data is shown for genotypes selected for distribution to collaborators

of the INCO project. Genotypes were classified as: HE: highly efficient, E: efficient, I: intermediate, LE: low efficiency.

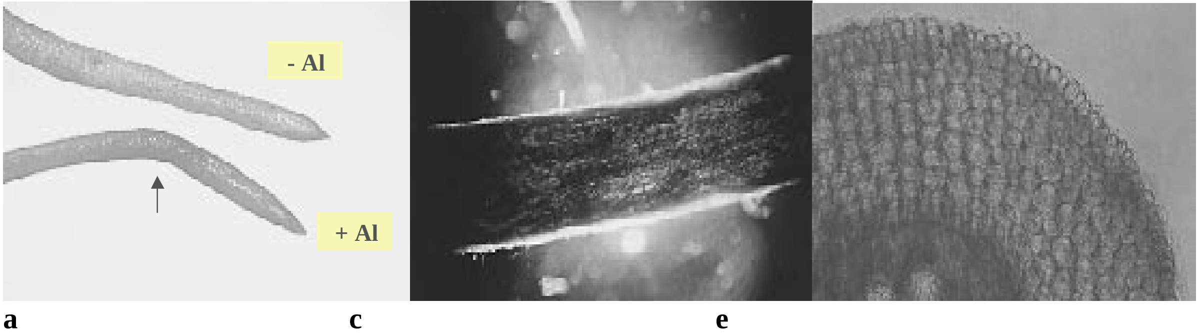
HYBRID	KG EARS 2 ppm P	KG EARS 15 ppm P	2/15 ppm	Classification
BRS 3060	10630 (1)*	11880 (3)	0.89	HE
HS 20 X 723	9006 (3)	10740 (10)	0.83	HE
HD 91102	7630 (23)	8384 (48)	0.91	E
HD 9176	7982 (15)	8926 (39)	0.89	E
BR 201	6780 (46)	7737 (58)	0.87	E
BR 201-F	6965 (42)	8472 (47)	0.82	E
HD 9481	7535 (31)	9915 (25)	0.75	I
BR 201-M	6840 (45)	9206 (36)		I
HS 64 X 1143	5596 (61)	7425 (60)	0.75	I
HS 11 X 723	6687 (50)	9146 (37)	0.73	LE
HD 9148	6750 (47)	10770 (9)	0.62	LE
HS 13 X 1143	4825 (63)	7030 (62)	0.62	LE
Mean of hybrids	7322	9436	0.77	
LSD 5%	1824	1657		

* ranking between the 64 hybrids evaluated at each P level

Activity 2 – Mechanisms of Al Tolerance and P Efficiency

Maize genotypes sensitive to Al toxicity accumulate considerable Al in the root tips while Al tolerant genotypes tend to exclude Al from root tips.

Hematoxylin reacts with Al forming a pigmented hematoxylin-aluminum complex. Hematoxylin staining of root tips of seedlings grown in a media with Al stress was used to classify seedlings for tolerance and susceptibility to Al toxicity. A study was conducted to verify the existence of spatial variation in the distribution of hematoxylin-aluminum complex in the root tips of maize and to verify the potential of using hematoxylin staining to classify seedlings for tolerance and susceptibility to Al toxicity. Typical results are demonstrated in Figure 1.



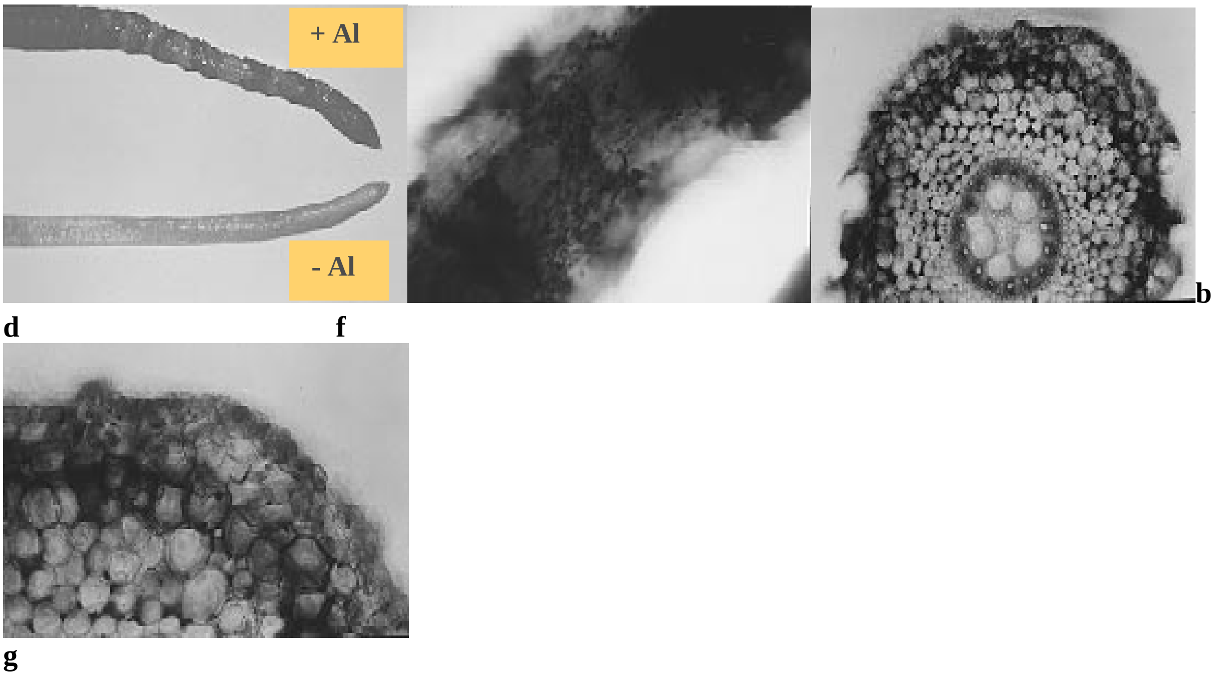


Figure 1. a - b Root tips of maize, stained with hematoxylin, after 24 hours of zero and 222 μmoles L^{-1} de Al. a: tips of L1143 (tolerant to Al toxicity) in the presence and absence of Al, with the arrow indicating the region of constriction stained by the hematoxylin, and b: tips of L53 (susceptible to Al Toxicity) in the presence and absence of Al (3x enlargement), c – d Detail of the epiderm of the maize roots stained with hematoxylin after 24 h de exposure to 222 μmoles L^{-1} de Al. c: root of L1143, detail of the region of the constriction; and d: root of L53. (Enlarged 25x), e – g Transversal cut of root tips of maize stained with hematoxylin after 24 h of exposure to 222 μmoles L^{-1} de Al. e: L1143, (enlarged 100x); f and g: L53, (enlarged 100x and 200x, respectively).

The staining of the roots of maize seedlings by hematoxylin after a period of Al stress in nutrient solution made it possible to see the spatial distribution of Al in the root tips. The staining technique was shown to be efficient in discriminating between Al tolerant and susceptible genotypes in a precise, rapid and nondestructive manner. In Table 3 the relative ratings between net seminal root growth (NSRG), relative seminal root growth (RSRG), and hematoxylin staining scores are presented for 23 maize genotypes. The phenotypic indexes for Al tolerance, NSRG and RSRG, frequently used in plant improvement programs were closely correlated with and hematoxylin staining scores. Correlations between RSRG, NSRG and hematoxylin staining scores are shown in Figure 2.

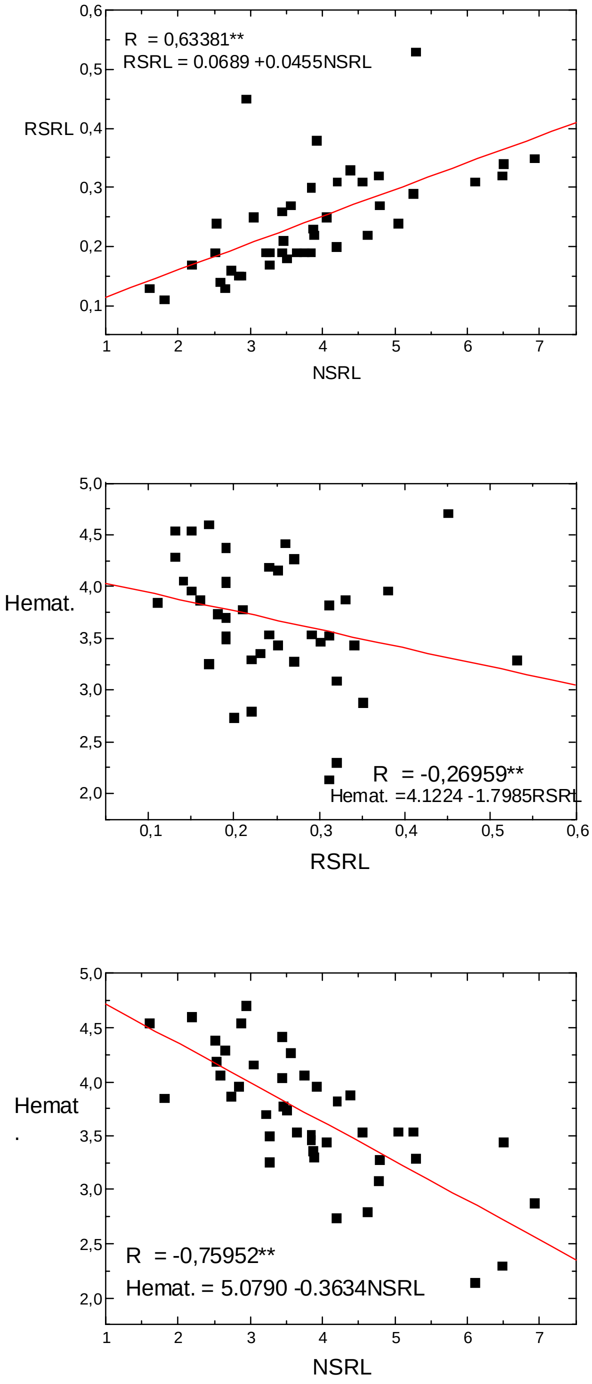


Figure 2. Correlation coefficients between means of NSRG (NSRL), RSRG (RSRL) and score of hematoxylin staining of the hybrids of a diallel cross of nine maize lines (significant at 1% T-test).

A series of controlled experiments were designed and conducted using contrasting genetic standards to identify mechanisms controlling tolerance to Al toxicity and improved phosphorus efficiency and to study mode of inheritance of these traits. In the early years of this program tolerance to Al toxicity was confounded with improvement in P uptake efficiency.

Table 3. Average values of NSRG, RSRG, and hematoxylin staining scores for maize genotypes compared to previous RSRG classifications made by Embrapa Milho e Sorgo.

RSRG			**NSRG**			**Hematoxylin Score**		
Genotype	Mean		Genotype	Mean		Genotype	Mean	
L188[T]	0,7449	a	L175[T]	6,950	ab	L29[S]	4,500	a
L46[T]	0,7254	a	L88[T]	6,693	abc	L98[S]	4,250	ab
L58[T]	0,6859	ab	L188[T]	6,167	abcd	L126[S]	4,083	abc
L88[T]	0,6822	ab	L58[T]	5,860	abcde	L33[S]	4,083	abc
L160[T]	0,6107	abc	L18[T]	5,857	abcde	L197[S]	3,583	abcd
L18[T]	0,5965	abcd	L1327[T]	5,763	abcdef	L201[S]	3,500	abcd
L45[T]	0,5530	abcde	L46[T]	5,570	abcdef	L229[S]	3,167	bcde
L175[T]	0,5234	abcdef	L160[T]	5,383	abcdef	L122[S]	3,083	cde
L21[T]	0,4933	abcdef	L45[T]	5,330	abcdef	L27[S]	3,000	cde
L124[S]	0,4117	bcdefg	L124[S]	4,453	bcdefg	L124[S]	2,833	de
CMS36[T]	0,4111	bcdefg	L21[T]	4,430	bcdefg	L160[T]	2,250	ef
L122[S]	0,3856	cdefg	L27[S]	4,380	bcdefg	L58[T]	1,667	fg
L197[S]	0,3567	cdefg	L53[S]	4,167	cdefg	CMS36[T]	1,643	fg
L16[T]	0,3561	cdefg	L126[S]	3,737	defg	L88[T]	1,583	fg
L27[S]	0,3317	cdefg	L98[S]	3,523	efg	L18[T]	1,250	fg
L98[S]	0,3298	cdefg	L33[S]	3,500	efg	L16[T]	1,250	fg
L201[S]	0,3101	defg	L122[S]	3,453	efg	L175[T]	1,083	g
L33[S]	0,2986	efg	L201[S]	3,427	efg	L21[T]	1,000	g
L29[S]	0,2648	fg	L197[S]	3,263	efg	L188[T]	0,9167	g
L53[S]	0,2595	fg	L29[S]	3,237	efg	L45[T]	0,9167	g
L126[S]	0,2505	fg	L16[T]	3,167	fg	L1327[T]	0,7500	g
L229[S]	0,1917	g	L229[S]	2,690	g	L46[T]	0,6667	g

Means followed by the same letter not significantly different, Tukey test $p < 0{,}05$.

[T] Genotype classified as tolerant to Al toxicity by Embrapa Milho e Sorgo

[S] Genotype classified as tolerant to Al toxicity by Embrapa Milho e Sorgo

The first commercial hybrid released by Embrapa, BR 201 as tolerant to Al toxicity was also shown to be more efficient in P acquisition more recently. This resulted in predicting that inheritance of tolerance to Al toxicity was multigenic and complex. We now understand that the inheritance for tolerance to Al toxicity is simpler and more straightforward. However, it appears that more than one mechanism is involved in Al tolerance in maize, and different tolerant genotypes may actually have different mechanisms or multiple mechanisms. The understanding of these mechanisms is very important in developing research and development strategies and priorities. During the past thirty months, the integrated team of researchers at Embrapa conducted studies of the basic mechanisms, at the physiological and genetic levels, that control the adaptation of maize to high levels of Al saturation and to a lesser degree to low levels of available P and P fixation in the soil. Figure 3 demonstrates that one of the traits associated with P acquisition efficiency is greater root growth of more efficient genotypes under P stress. This research approach, integrating the existing genetic resources and acid soil management practices, with basic knowledge in plant physiology and molecular biology has accelerated the process of developing improved high yielding cultivars for improving sustainable maize production systems in acid soil ecosystems.

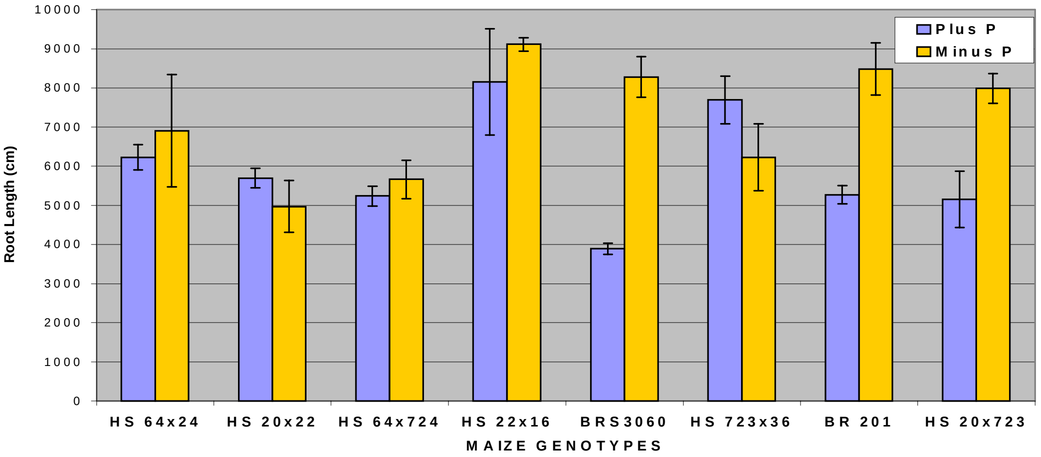
Research on acid soils of the cerrado has shown that as the moisture supply in the soil profile is depleted various changes occur in the availability and concentrations of P and N. The soils of the cerrado generally have low water holding capacity; water use by the maize plant is high at flowering, and during the grain filling stages of development. Rainfall distribution during this period may also be sporadic and irregular, leading to a scenario of repeated wetting and drying of the soil profile. As the soil dries, phosphate concentration in the soil solution approaches zero, NO3-N concentration is reduced and the NH_4-N/NO_3-N ratio increases.

Cultivars unable to cope in this scenario will produce poorly, but those that are more resilient and can temporarily endure repeated cycles of low P levels and utilize the NH4^{+}-N more efficiently should have greater and more stable production. Several studies have been initiated to help better understand what occurs in this type of scenario. Research has been conducted to identify molecular markers for tolerance to Al toxicity and more recently, research has been initiated to develop and characterize genetic materials and to identify molecular markers for P uptake efficiency.

Research is currently underway at Embrapa to evaluate organic acid exudates of maize roots under both P and Al stress, principally citrate and malate, with the objective of understanding the effect of these molecules on tolerance to toxic Al and the efficiency of P absorption by the root. Currently, new procedures are being evaluated to improve the efficiency of qualifying and quantifying these organic acids in complete nutrient solution. High affinity phosphate transporter probes have been obtained to study the induction of maize high affinity phosphate transporter genes under P stress in the genetic maize standards for P uptake efficiency.

The understanding of mechanisms that regulate the tolerance to nutrient stress or improved plant nutrition in this acid soil complex, at the plant, cell and molecular levels; and the development of genetic markers is expected to accelerate the breeding program of developing high yielding cultivars better adapted to the acid soil complex of the tropical acid savannas of the world.

Figure 3. Root length of contrasting maize genotypes for P efficiency with six days of P omission.



The results from this project indicate that plants apparently have the ability to alter the availability and/or uptake (transfer across membrane from the soil to inside the root) of phosphate in the rhizosphere. Additionally, research results to date indicate that a major tolerance mechanism to Al toxicity is the exudation of organic acids (OAs), principally citrate and possibly malate into the soil rhizosphere that can chelate with and detoxify Al. Other research also indicates the sorghum roots exudate oxalic and succinic acid under P stress and these OAs may release fixed Pi from the soil in the rhizosphere, increasing Pi acquisition efficiency. Additionally, it has been recently found that both phosphatase levels in sorghum roots and high affinity Pi transport genes in both maize and sorghum are induced under Pi stress. The identification and analysis of physiological and molecular determinants for tolerance to Al toxicity and P efficiency, regulation of gene expression controlling these traits, and the utilization of genomics information is expected to enhance the efficiency of selection of genotypes in crops such as maize.

The findings of this project may also be useful in developing research strategies and improved cultivars or other species better adapted to marginally fertile soils. The final outcome will be an increase in food security in developing countries of the tropics where food production has frequently been inadequate to meet the needs of its people.