Summary

The main objective of the project was the genetic improvement of maize for a more successful incorporation of maize into cropping systems on acid soils with high aluminium (Al) supply and low Phosporus (P) availability in Sub-saharian Africa, in South America, and the Caribbean. The better understanding of the physiological and molecular mechanism leading to acid soil resistance in general and Al resistance and P efficiency as principal requirements for adaptation to such soils, specifically, will lead to the development of quick screening techniques thus facilitating and enhancing the progress made by breeding. Parallel to the genetic approach, the development of agronomic technologies and the quantitative description of the processes at the soil root interface and of crop growth on acid soils will contribute to the development of natural resource-friendly, sustainable, and economic cultivation of maize on acid soils.

The objectives of **workpackage 1** were to develop germplasm with improved adaptation to the acid soils and to develop more efficient breeding technologies. Heterosis contributes substantially to grain yield in acid soil environments **(INRA, IRAD, CIMMYT, EMBRAPA).** Additive and dominant gene actions were equally important for hybrid performance. Development of non-conventional hybrids between tolerant-source populations from different origins could be a promising avenue towards increasing maize yield in cropping systems on acid soils. The mode of inheritance of Al resistance was predominantly through additive gene action **(UHANN)**. Results on both populations and inbred lines clearly show the potential of using Al-induced callose formation as a selection trait for adaptation to acid, Al-toxic soils **(UHANN, IRAD, INRA).** High correlation between *per se* value and general combining ability could greatly simplify the breeding schemes. Field results showed that although Al resistance is necessary to ensure plant growth in acid soils, other factors such as the transfer of nutrient to leaves and a higher RUE may partially compensate for a moderate sensitivity to Al. N may play a major role, together with P uptake **(INRA)**. Inbred lines with more efficient or/and more responsive to P were identified and the phenotyping protocol was optimised **(EMBRAPA)**. Target selection traits quantifying root and leaf responses to stress and nutrient indices were determined **(INRA)**. Sets of genetic material fitting to genetic dissection of tolerance were developed. F1 diallel crosses are available for collaborative genetic and physiology combined studies. Several of these materials have been the main source in cultivars development and release during the latest years **(IRAD, EMBRAPA, INRA)**.

The Agronomic research in **workpackage 2** aimed at developing improved soil management methods in conjunction with adapted maize cultivars, in order to make maize based cropping systems on acid soils more sustainable. Experimental sites were near Yaoundé in Cameroon (**IRAD)** and near Villavicencio in Colombia **(CORPOICA**), on acid, Al-toxic soils. The Cameroon site is in the mid-altitude forest zone with extensive systems on a mosaic of cultivated patches. The Colombia site is in the Eastern, grassy plains that are rapidly being transformed from extensive pastures to intensive, fully mechanized, maize-based systems. Five different experimental approaches were used, (1) a long-term fertility trial dating back 6 years on the direct and residual effects of lime and organic manure (OM) amendments at both sites, (2) a maize-legume rotation experiment using different lime rates in Cameroon, (3) tests of mycorrhiza inocculum effects on maize yields in Cameroon, and (4) studies on the management of root system development under soil acidity and compaction constraints. Comprehensive results of soil analyses and cropping systems modelling using STICS (5) are not yet available because analyses are still in progress. The results permit the following general conclusions: (i) At both sites in Cameroon and Colombia, lime application, in combination with growing an acidity-tolerant maize cv, was essential to sustain high yields and gave strong residual effects even 3 years after application. (ii) Chicken manure was also effective, whereas green manure incorporation disappointed. (iii) 3-year rotation of maize with cowpea had no advantage over the traditional fallow/burn practice, and rotation with *Mucuna* reduced maize yields for unknown reasons. (iv) Soil acidity reduced root system depth, thereby increasing drought stress. Liming was effective in increasing root depth in Cameroon but not in Colombia due to compaction. (v) Compaction and acidity have independent, multiplicative effects on root growth. (vi) Mycorrhiza inoculation of the soil had moderate but positive effects on yield in Cameroon. These results should be followed up locally

by (1) adaptive research in order to fine tune recommendations while taking into account returns to investment and farmers' resources, and (2) by modelling activities aiming at extrapolating the results to other environments and climatic scenarios (drought risks associated with root system depth).

Investigations in **workpackage 3** show that enhanced citrate synthesis and enhance citrate release from root tips are efficient mechanisms for Al resistance in maize (**UEC, EMBRAPA**). Anion channels seem responsible for Al-induced citrate release. By expressing a citrate synthase gene in a subtropical maize genotype, it is possible to generate lines with increased Al resistance as assessed under the experimental conditions used in this work (**CIEC)**. In addition to citrate exudation from root tips other mechanisms may be implied, especially in the extremely Al-resistant maize variety Cateto. Al resistance in maize can be associated with exudation of flavonoid type phenolics from root tips, a better maintainance of vacuolar malate levels, and changes in a few low molecular weight proteins (**UAB**).

The objective of **workpackage 4** was to contribute to a better understanding of the mechanisms that are involved in the efficiency of P uptake and utilization which is crucial to develop improved varieties for maize production in acid tropical soils. The efficient response of tropical maize varieties to P fertilizer supply is based on enhanced P uptake-efficiency. Enhanced P uptake-efficiency in maize is achieved by P deficiency-induced release of citrate into the rhizosphere soil and by the maintainance of elevated rates of phosphatase release in presence of low P substrate concentrations (**CIEC, UAB)**). Based on this information transgenic maize lines that overproduce citrate were produced. These transgenic lines were found to be more resistant to exposure to toxic concentrations of Al and to have a higher capacity of P mobilization when grown in soil containing low available P. Among the P-efficient lines selected by **EMBRAPA**, more favourable root architecture and efficient AM symbiotic relationships appeared more important than the release of organic acid anions.

There is increasing evidence that cell-wall characteristics may modulate Al sensitivity. In **workpackage 5**, additional evidence for this assumption using different experimental approaches was achieved. Cell-wall pectin is the main binding site of Al in the cell. The results indicate genotypic differences in pectin contents and its degree of methylation, however, their relationship to genotypic Al sensitivity is still not yet clear. The use of pectin-specific antibodies for the localization of specific pectins differing in degree of methylation in the root apex appears promising in relation to Al localization in the root tissue (**UHANN)**. Silicon treatment leads to the formation of hydroxyaluminiumsilicates (HAS) in the apoplast of the root apex thus detoxifying Al (**UHANN**). The comparison of Al rhirotoxicity at low and high substrate pH produced circumstantial evidence that at bulk solution pH 8.0 the maintenance of an acidic apoplast leads to the formation of cationic Al hydroxy species and $Al(OH)_3$ inducing root-growth inhibition but less plasma-membrane and cell damage than Al^{3+} dominating at low solution pH (**UHANN**). The expression of the *Zmal1* gene in transgenic tobacco plants only marginally improved Al resistance (**UEC**). This may be because this gene does not work properly in a heterologous environment. The α1-tubulin gene promoter has been shown to be the most adequate promoter to express genes coding for cell-wall characteristics (**CSIC**). Preliminary results indicate that expansin is expressed in maize root tips, which makes expansin a good candidate to test their role as cell wall-relaxing proteins under Al stress. The availability of transgenic plants specifically modified in cell-wall composition using root-specific promoters will be a powerful tool to relate specific cell-wall proteins to Al resistance.

In **workpackage 6** the role of the plasma membrane properties in the expression of Al resistance was evaluated. Unexpectedly, the expression of a sphingolipid desaturase gene from *Arabidopsis thaliana*. rendered maize plants more Al-sensitive (**UHANN**). Using *Arabidopsis* mutants differing in their fatty acid composition the assumption that an increase in Al resistance is achieved through an increase in unsaturated fatty acids in the membrane could be confirmed (**UHANN**). The results of the study on oxidative stress enzyme activities (**EMBRAPA, UEC**) indicate that Al causes oxidative stress only after a sustained inhibition of root growth. It is concluded that protein oxidation and not lipid peroxidation is the major short-term effect of the oxidative stress caused by Al. **UEC** expressed the *OMT134* gene under the control of a new construct, based on the *Zmal1* promoter. This gene is expressed only in the epidermal cells of the root tip. The transformation of maize plants with a

new construct named pZ123-OMT-BAR is underway. Transgenic tobacco plants expressing the *Zmgst2* gene encoding a glutathione-S-transferase **s**howed only a marginal effect on Al resistance. Transgenic maize plants using the same construct are being produced and evaluated.

In **workpackage 7**, **EMPRAPA** aims at identifying genomic regions associated with Al resistance. An efficient methodology to evaluate Al resistance in maize using a root index measured in nutrient solution containing toxic Al has been developed. Five RFLP markers closely associated with Al resistance that explain 34% of the phenotypic variance have been identified in an early cycle, $F_{3:4}$, of Recombinant Inbred Lines (RILs) developed from a cross of contrasting inbred lines for Al resistance. Five QTLs associated with Al resistance were mapped on chromosomes 2, 6 and 8, explaining together with the background markers 60% of the phenotypic variation. QTL4 was mapped at the chromosome 8 close to the gene idh1 coding the isocitrate dehydrogenase, and marker umc043 explaining close to 11% of the Al resistance by multiple regression analysis was located flanking the malate dehydrogenase (mdh5) locus. These genes encode for enzymes involved in the organic acids pathways, which has been one of the most studied mechanism for Al resistance in plants. One hundred and sixty-eight RILs segregating for Al resostance have been advanced to the $F_{6:7}$ cycle. These lines will be phenotyped and used to test new RFLP and SSR (230) markers flanking the genomic regions previously identified as related to the character.

In **workpackage 8, EMBRAPA** examined the expression of high affinity phosphate transporters (PT) in 8 maize lines from EMBRAPA, which are considered as standards for P efficiency under P stress, using the *Zea mays* PT gene (ZmPT1-6) as probes. Once there was no difference in the gene expression among the lines studied we expanded our work utilizing the promoter of a PT gene, AtPT2 from *A. thaliana* to direct the citrate expression into the maize roots under conditions of P stress, aiming at increasing the acquisition of soil mineral-bound phosphate. The analysis of transgenic maize plants resulted in harboring the promoter of the AtPT2 gene which drives the GUS reporter gene expression in the root growth zone particularly under P stress. Now, maize transgenic plants containing the AtPT2 promoter directing the expression of citrate synthase-gene are being generated.

The objective of **workpackage 9** was to identify promoters that are preferentially expressed in the root apex. This is essential to analyse the role of Al-induced genes. UEC has isolated *Zmal1* and *Zmgst2* genes and CSIC has isolated three *α-tubulin* genes. The promoter sequence of these genes have been obtained and fused to the *GUS* reporter gene to test their tissue specificity in transgenic plants. The promoters from Zmal1 and Zmgst2 genes (both Al-induced and expressed in the root tips) were evaluated in transgenic tobacco and *Arabidopsis* plants, but they failed to be recognized by the heterologous transcriptional machinery. Studies using transgenic maize plants are underway. The *α1-tubulin* gene promoter has a root-specific expression, is detected in maize root tips, and is evaluated in model systems. Moreover, tissue-specificity of the *α1-tubulin* gene promoter is conserved between monocots and dicots as evaluated by CSIC. This makes the *α1-tubulin* gene promoter a powerful tool to express Al-induced genes in monocots and dicots and to dissect mechanisms of Al resistance. Using micoarrays, UEC has initiated a large-scale detection of genes that are modulated by Al-stress in Al-resistant line Cat100-6. A number of genes that are regulated by Al stress are defined and their expression pattern is being confirmed.

The objectives of **workpackage 10** were to write (**INRA**), to calibrate (**CIRAD**) and to validate (**CIRAD, CORPOICA, IRAD**) the model for Al-H-P interactions in acid soils, with emphasis on the interactions between H, Al, P and organic anions released by roots. The final objective was a substantial contribution to the comprehension of the functioning of the rhizosphere in the acid soils and the interactions between Al-H-P. The writing of the model consisted of three points: i) the adaptation and harmonisation with the new Windows environment, ii) the writing of a module of anion adsorption, iii) the writing of a model with anion exchanger. The calibration and the validation of the model concerned (i) the effect of citrate and of soil pH on the dissolution of P, Al and Fe, (ii) the fixation of citrate by the soil, and (iii) the definition of kinetics and equilibrium constant for Al and P. The model used made it possible to analyse the dynamics of the elements H, Al, Ca and P in the rhizosphere of the soils of Cameroon and Colombia and to simulate variations of coherent conditions

of medium with the agronomic observations carried out on the two studied sites. The role of citrate as a ligand of Al in solution is well highlighted. The influence of the excretion of citrate on the dynamics of P is shown but the description of the effects of the simulated citrate fluxes remains weak.

In **workpackage 11, CORPOICA** isolated and characterised michorrizal arbuscular (MA) fungi spores in association with two maize cultivars Sikuani and Clavito grown an acid soil of the Colombian Eastern Plains. High relation coefficients between soil conditions (pH, organic matter %, P, K, and Al%) and MA fungi populations were found. With the use of specific primers, spores isolated from *Glomus*, *Entrophospora* and *Gigaspora* were identified. **EMBRAPA** found that fingerprints of the mycorrhizal populations were generated by denaturing gradient gel electrophoresis (DGGE), and VAM amplified DNA fragments were separated based on nested PCR. The mycorrhizal-DGGE profiles reflected population differences in the mycorrhizal community in the rhizosphere of P-efficient and P-inefficient maize genotypes. **UHANN** studied the interaction of soil conditions and AM species under controlled conditions. AM infection clearly enhanced shoot and root growth under P-limiting conditions. Among the AM fungal species *Acaulospora* and *Glomus* were more effective than *Entrerospora* under less severe stress conditions (+P,+lime). Under severe stress conditions (-P, -lime) only *Glomus* clearly stimulated maize growth.