Molecular studies for linkage analysis and determination of quantitative trait loci (QTL) for acid soil tolerance in maize (*Zea mays* L.)

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Plant Breeding

Major Professor: Dr. Arnel R. Hallauer

Iowa State University

Ames, Iowa

2000

Graduate College
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I want to dedicate this work to my great family.

To Martha Elena my “compañera” for her love, patience, comprehension and confidence to share with me a happy future full of love.

To Juan Sebastian and Nicolas whom are the prolongation of our lives as proud parents.

To Leonila and Alejandro, my parents, for their love, guidance and support.

To my sisters and brothers, **Gloria, Olga, Jairo, Martha, Adriana and Santiago** for their love and friendship.

To Rosita, my mother in law, for giving me her most precious treasure and for her love and support.

To the memory of **Luz Marina** †, my sister in law, for her happiness and her sincere way of defining life.

To Rodrigo, Guillermo and Angela for their fraternity and friendship.

To my friends **Jorge Gutierrez, Jhon Ospina † and Luis Javier Cifuentes**†, I will not forget you.

With all my love, God bless of all you.

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ACKNOWLEDGEMENTS

This project could have not been possible without the help, support, and friendship of the people that were with me from the beginning until the end.

I would like to thank Dr. Shivaji Pandey for building the idea of this project and for giving me the opportunity to develop and run his idea. He has given me his trust and friendship for the past 16 years. Thanks to Jean Marcel Ribaut for his scientific guidance and his friendship. To Mateo Vargas and José Crossa for their orientation, comments and support on the statistical analysis. Special thanks to Eva Huerta who was very patient when she taught me the lab protocols. Elba Magaña and Margarita Crosby whom helped me with all the lab analysis.

In **CIMMYT** Colombia I would like to specially thank Dr. Carlos De Leon, Dr. Luis Narro, Ing. Maria Del Pilar Arias, Ing. Freddy Salazar and Dr. Juan Carlos Perez for their excellent field work, comments and support.

In **CORPOICA** I want to show my gratitude towards Dr. Rafael Aubad, Dr. Alvaro Uribe, Dr. Ricardo Torres, Dr. Juan Jaramillo, Dr. Laura Rugeles, Dr. Javier Narvaez and Dr. Andres Laignelet for their administrative support and for encouraging me to continue my Ph.D. studies.

In **CORPOICA Regional 2** my most sincere thanks to Dr. Beatriz Escobar, Dr. Sony Reza, Ing. Sergio Mejia, Ing. Ramiro Urrea, Ing. Jose G. Ospina and to everyone who plays a part in the Turipana Research Center.

In the Colombian, Francisco José de Caldas, Institute for the Development of Science and Technology (**COLCIENCIAS**), I show my gratitude towards Dr. Fernando Chaparro, Dr. Hernan Jaramillo and Dr. German Urrego, for their administrative and economical support with the project's and scholarship's budget throughout the past four years.

In **Iowa State University** I would like to thank Dr. C. Martinson, Dr. L. Pollak, Dr. T. B. Bailey and A. R. Campbell for being part of my committee and for being role models in my career. Finally I would like to specially thank Dr. Arnel R. Hallauer for giving me advice, guidance, knowledge, trust and friendship.

GOD BLESS YOU ALL!

ABSTRACT

Acid soils include approximately 4 billion hectares of the earth's surface. Soils with pH < 5.6, deficiency of calcium, magnesium, phosphorous, molybdenum, iron, and Al saturation >35% with P level <16 parts per million are considered acidic for maize (*Zea mays* L.). Because of acid soils, fewer and smaller roots are produced, reducing the plant's capacity to uptake water and nutrients from the soil. The objectives of this study are to develop the marker linkage map for an acid soil tolerant maize F_2 segregating population, to dissect the quantitative trait loci (QTLs) for several traits, and to determine whether these QTLs could be used in a marker-assisted selection program for acid tolerance in maize. An F_2 population of 221 individuals was genotyped at 118 SSR loci and 214 F_3 families were evaluated in an alpha lattice design (22x10). The trials were grown in five environments, three acidic (V55, V65, and AYA65) and two normal fertile (PAL and TUR) in Colombia. Female flowering, male flowering, anthesis-silking-interval, yield, ears per plant, and plant and ear height were measured. Except for male and female flowering and anthesis-silking-interval at AYA65, acid soil environments tended to have less the genetic variance. The estimates of heritabilities (h^2) in acid environments were generally lower for all traits but yield in V65 (36%) and AYA65 (60%). The total length of the SSR linkage map was 1836.2 cM with a mean density of 15.56 cM. For all traits evaluated and based on the composite interval mapping analysis (LOD=2.5), there were 66 QTLs identified for each environment. Thirteen QTLs were detected across acidic soils, 33 across normal-fertile soils, and 40 QTLs across all environments. In this study, no QTL with major effects were identified. QTLs had low single and total R^2 value for individual environments and across environments.

INTRODUCTION

Maize (Zea mays L.) is a major food crop widely grown in Colombia, and it is grown on about 700,000 hectares each year. In 1996 the national average maize grain yield for Colombia was 1.87 t ha-1 and in the same year, Colombia imported about two million tons (FENALCE, 1995). According to the International Maize and Wheat Improvement Center (CIMMYT, 1992) the developing countries have an average grain yield of 2.4 t ha^{-1} vs. the developed countries with 6.7 t ha^{-1}. One of the reasons for these yield differences is that more than 80% of the maize in the developing countries, including Colombia, is grown in tropical soils with poor fertility (CIMMYT, 1992).

Among many others, some of the causes for the low soil fertility are low organic matter content (high mineralization rate), aluminum (Al) toxicity (saturation > 35%), manganese (Mn) toxicity, and high phosphorus fixation rate. Soils with pH < 5.6, deficiency of calcium (Ca), magnesium (Mg), phosphorous (P), molybdenum (Mo), iron (Fe), and Al saturation >35% with P level <16 parts per million are considered acidic for maize. These soils are acidic because the parental material was acidic and low in basic cations or simply because these elements were removed by leaching or harvested crops (Duque-Vargas *et al*., 1994; Granados *et al*., 1993; Aldrich *et al*., 1975).

Acid soils include approximately 4 billion hectares of the earth's surface. From this total, about 41% are in the America continent, 26% in Asia, 17% in Africa, 10% in Europe, and 6% in Australia and New Zealand. Only 4.5% of this area is under arable crops, and the remaining areas are under forest (67%), savannas and prairie vegetation (18%) and perennial tropical crops <1% (Salazar *et al*., 1997).

Sanchez, (1977) estimated that acid soils in 48 developing countries involved 1.7 billion ha. Acid soils represent approximately 43% of tropical area in the world: 64 % of tropical South America, 38% of tropical Asia (mainly in Indonesia, Thailand, Malaysia, India, China, and The Philippines), 27% of the tropical Africa (specially in Ivory Coast, Zaire, Zambia, Tanzania, Uganda, and Zimbabwe), and 10% of Central America, Caribbean, and Mexico (Granados *et al*., 1993). In South America (Brazil, Peru, Colombia, Venezuela, and Ecuador) about 80% of the agricultural activities occur in acidic areas. Brazil has the largest area with 205 million hectares (Cerrado Brasileiro) of which 112 million are suitable for agricultural production (Torres, *et al*., 1997).

Colombia is beginning to explore its " Llanos Orientales" (eastern plains). This area is composed by acidic savannas with about 17 million hectares that are suitable for potential agricultural production. In the future, the goal is to incorporate these lands within the national agricultural area. The "Llanos" have a large quantity of fine soils, caolinitics, and isohiperthermics. About 75% of the area is classified as Oxisols, 10% as Ultisols, and 15% in other categories. These soils have low organic matter, pH, P, Ca, Mg, K, B, and other nutrients, and high interchangeable aluminum (Al). In general these soils are considered very good soils for their physical conditions but with limitations in their chemical conditions. The majority of the "Llanos" is used for extensively for livestock. Annual crops, such as rice, soybean, cowpea, etc., have been successful when they are grown near processing centers, but they are not economical activities when they are grown far from the processing centers.

Maize is an attractive option for the Llanos' farmers. Maize could be consumed by humans and by animals in the same areas of production. Maize has not been planted

extensively on the "Llanos" because acidic tolerant varieties are not available. Researchers have estimated that maize is currently planted in 8 to 20 million hectares of acid soils each year. This area is consists of 2.5 million hectares of maize in Asia, 1.5 million hectares in Africa, 1.0 million hectares in Central America, Mexico, and the Caribbean, and 3.0 million hectares in South America (Granados *et al*., 1993; von Uexkull and Mutert, 1993).

Maize is considered one of the most susceptible crops to acid soils, more than wheat and rice. Maize is either similar to or slightly more susceptible to acid soils than sorghum, cotton and soybean (TropSoils, 1991; Tan, 1993). Because of acid soils, fewer and smaller roots are produced by the maize, reducing the plant's capacity to uptake water and nutrients from the soil. As the soil acidity increases, this reduces the survival and function of the micro-organisms that are responsible for mineralization of organic matter and subsequently limits the availability of nitrogen, phosphorus, sulfur, and many micro-nutrients to crops (Foy, 1976). Unfortunately, the increasing CO_2 level in the atmosphere and more applications of ammonium-based nitrogenous fertilizers (especially in the developing countries) are two factors that tend to increase the problem of acid soils.

At this time, basically two strategies have been used to increase the maize yields in acid soil. First, the application of lime amendments has been a reliable treatment for correcting soil acidity, but it is not an economic option for farmers who live far from lime sources and do not have funds to purchase lime. Liming is an agronomic practice demanding high mechanical power especially at the sub-soil level (deeper than 30 cm), and it must be repeated every 3 to 4 years, but this is not a permanent solution to the problem. Additionally, this agronomic practice is clearly incompatible with sustainable production (conservation tillage and low input technologies). The second strategy has been the development of

genetically acid tolerant maize cultivars and sustainable agronomic practices, which offer another solution to the problem. The second strategy is environmentally clean, energy conserving, and permanent. Acid tolerant cultivars are relatively inexpensive to develop and easily accessible to the poor farmers (Salazar *et al*., 1997; Pandey *et al*., 1994; Granados *et al*., 1993; Pandey and Gardner, 1992; Foy, 1976).

In order for plant breeding to achieve success in the development of acid soil tolerant cultivars it requires 1) presence and knowledge of gene action controlling tolerance (genetic variation), 2) techniques that are reliable and efficient in laboratory and field to differentiate between tolerant and susceptible genotypes, and 3) appropriate plant-breeding methods.

Researchers have reported genetic variation for tolerance to acidity in maize and improved germplasm is available (Bahía Filho *et al*., 1978; Rhue *et al*., 1978; Magnavaca, 1982; Miranda *et al*., 1984; Furlani *et al*., 1986; Lopes *et al*., 1987; Kasim *et al*., 1990). Quantitative inheritance studies for tolerance of maize to acidity and Al toxicity have been reported by Sawazaki and Furlani (1987), Magnavaca *et al*., (1987), Lima *et al*., (1992), and Duque-Vargas *et al*., (1994). On the other hand, Rhue *et al*. (1978) and Miranda *et al*. (1984) reported qualitative inheritance for Al toxicity.

In quantitative inheritance studies, Magnavaca *et al.,* (1987), Naspolini *et al.,* (1981), and Pandey *et al*., (1994), showed that genetic variance for yield in acid soils was mainly additive, while Duque-Vargas *et al*., (1994) and Borrero *et al*., (1995) reported that dominance variance was equal or more important. Pérez and Lopes de Souza Jr. (2000) studied eight related and non-related inbred S_6 lines of maize possessing different levels of tolerance to acid soil. Using the generation mean analysis they found that the dominant genetic effects were more important for the grain yield than the other types of genetic effects.

One of more common techniques used in studies of the tolerance to soil acidity in maize is based on the effect of Al on seminal root growth in nutrient solution. Although Al toxicity is the most important factor in acid soils, there are other toxicities, deficiencies, and interactions with the organic matter content that constitute the complex responsible for lower maize yield (Evans and Kamprath, 1970; Adams and Moore, 1983; Torres, *et al*., 1997).

Studies conducted at CIMMYT reported that additive genetic correlation for yield among acidic sites and between acidic and non-acidic sites were small but generally positive. These studies showed that general combining ability was highly significant, accounting for 89% of the genotypic variation, and specific combining ability was non-significant for yield. Heritability, estimated using half-sib family means, averaged 38% for yield. Although grain yield showed the highest positive additive genetic correlation (0.84**) with ears per plant, direct selection for yield was more effective for improving yield under acidic soils. The magnitudes of additive, dominance, and additive x environmental variances and of additive genetic correlations among the environments suggested that recurrent selection in heterotic populations, based on multi-location testing, would be effective in improving grain yield of varieties and hybrids to be grown in acidic soils (Duque-Vargas *et al*., 1994; Pandey *et al*., 1994; Borrero *et al*., 1995).

Studies conducted in Colombia and other places suggest that recurrent selection methods have been effective in improving maize yield on acidic soils. Magnavaca *et al*., (1987) reported that the population “Composto Amplo”, after four cycles of half-sib selection had significant yield improvement. Lima *et al*. (1992) observed an average change of 15.1% after two cycles of positive and negative selection for acid soil tolerance.

CIMMYT and several national programs (NARS) in the developing countries have developed and improved six maize populations of different grain color and texture for tolerance to soil acidity (SA3, SA4, SA5, SA6, SA7, and SA8). The basic plant-breeding method used for yield improvement has been evaluation of either full-sib or S_1 families at different levels of Al toxicity and P deficiency. Some results of this collaborative work are presented. Granados *et al*. (1993) reported in the SA3 population yield improvement of 2% per cycle after 14 cycles of modified ear-to-row and 14% per cycle after two cycles of full-sib selection under acidic soils. These results showed the advantages to use either full-sib or S_1 families instead of modified ear-to-row at the CIMMYT program for acid soils in South America. Ceballos *et al*., (1995), in five populations reported yield selection gain on two acidic and one nonacidic environments, averaged 4.72% per cycle.

Recurrent selection has resulted in development and release of some acid- tolerant maize cultivars in the developing countries. The first acid tolerant maize variety released in Colombia, ICA Sikuani V-110, was developed from a recurrent selection program from the SA3 population. Also, three tolerant hybrids and several varieties have been released for acidic soils in Brazil and one variety in Indonesia.

Several studies were conducted in Colombia before ICA Sikuani V-110 was released in July 29, 1994. One of these studies compared one acid soil tolerant variety (SA3, later renamed as ICA Sikuani V-110) and one of the most non-acid tolerant maize germplasm in the tropics (Tuxpeño). Across 25 sites and three experiments conducted during 1992-1993 in a wide range of acidities, the yield of the tolerant variety ranged from 96 to 1500% of the susceptible variety, Tuxpeño, and averaged 153%. The superiority increased as stress

increased. Across five non-acidic sites, the yield of the tolerant variety ranged from 104 to 134% of Tuxpeño and averaged 111% (Granados *et al*., 1994).

Although some information on genetics of tolerance to Al toxicity is available (Granados *et al*., 1993) and several cultivars have been released, progress has been slow, and it has resulted from extensive and expensive field experimentation. Data from trials in acidic soils are characterized by high experimental error, which reduces heritability estimates and gains from selection (due to increased environmental effects). While field testing for yield evaluation of genotypes is indispensable, more precise information on physiological mechanisms responsible for tolerance to the acid soil complex and better screening techniques at plant and family levels would make research to develop tolerant cultivars more focused and efficient.

Biotechnology offers some techniques, such as restriction fragment length polymorphisms (RFLPs) and isozyme markers that have been used to develop genetics maps. Paterson *et al*., (1988) used RFLPs in tomato to detect quantitative trait loci (QTLs), which allowed the dissection of quantitative traits into their Mendelian's components. Several authors, using RFLPs, had worked with traits in maize such as thermotolerance, low-phosphorous stress tolerance, plant height, resistance to second-generation corn borer, and several morphological and grain yield components of F_2 segregating populations and recombinant inbred lines (Ribaut *et al*., 1996; Veldboom *et al*., 1994).

Today, we have molecular technologies that are more powerful, such as AFLPs (amplified fragment length polymorphisms), RAPDs (random amplified polymorphic DNAs), STS (sequence-tagged site), and Microsatellites (SSR, simple sequence repeats). The Polymerase Chain Reaction (PCR-technology) discovered in 1985, and the high level of

polymorphism for simple sequence tandem, demonstrated in 1989, have made possible a more rapid development for the genetic mapping and linkage analysis (Hearne, *et al*., 1992). PCR-technology basically involves a short DNA segment that can be amplified from a template *in vitro* using DNA polymerase and temperature cycling. The PCR reaction is highly specific, easily automated, and capable of amplifying minute amounts of DNA samples. PCR-technology has allowed for new and reliable markers that are now available (Ribaut and Hoisington, 1998).

"Simple sequence repeats (SSRs) or Microsatellites are composed of tandem repeats of two to five nucleotide DNA core sequences such as (AT)n, (GT)n, (ATT)n, or (GACA)n spread throughout eukaryotic genomes. The DNA sequences flanking SSR are generally conserved within individuals of the same species, allowing the selection of PCR-primers that will amplify the intervening SSR in all genotypes. Variation in the number of tandem repeats, n, results in different PCR product lengths. These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units. Different alleles can be detected at a locus by PCR, using conserved DNA sequences flanking the SSR as primers" (CIMMYT, 1996 p I-24). According to Ribaut *et al*., 1997b, " Such markers should map to specific loci, irrespective of which segregating population is used for mapping. If such loci are linked to genes involved in the expression of a trait of interest, it is possible, therefore, to use the corresponding markers for selection of the trait"(p 155).

For marker-assisted selection to be effective, a highly saturated marker linkage map is necessary (Dudley, 1993). The utility of molecular markers and marker-assisted selection scheme efficiency to transfer single target region or gene has been reported for several plants

(Ribaut and Hoisington, 1998). Modern biotechnology, at this time, has recognized the advantages of the partnership of the molecular geneticists with the plant breeders in order to develop new breeding schemes that could develop more and better cultivars.

Final goal of this project is to develop maize cultivars and hybrids, with accompanying agronomic technologies and cropping systems, for sustainable and economic production of maize in the Colombian acid savannas and another acidic tropical areas. Theoretically, molecular studies of acid soil tolerant maize cultivars would reduce the importance of experimental error, of environmental variance, and of genotype by environmental interaction to develop acid tolerant cultivars. The selection of acid tolerant genotypes could be more efficient with the use of molecular markers to aid in selection.

The specific objectives of this study are 1) to develop the marker linkage map for an acid soil tolerant maize F_2 segregating population, 2) to dissect the quantitative trait loci (QTLs) for several traits in a F_2 segregating population, and 3) to determine whether these QTLs could be used in a marker-assisted selection program for acid tolerance in maize.

MATERIALS AND METHODS

Plant materials

In 1995, 783 S_4 and 755 S_6 yellow and white lines were evaluated in two environments: normal-fertile and acid soils. In 1996, six tolerant and six susceptible lines from heterotic populations were selected (Table1). The crosses between tolerant and susceptible lines were done according with the heterotic group (Table 2).

In 1997, the 12 lines were evaluated, "*per se*", in three locations: one in normal-fertile soil (Palmira) and two in acid soil (Matazul and Villavicencio1). The results are presented in the Table 3.

Table 1. Acid tolerant and acid susceptible maize lines (S_4 and S_6) from heterotic populations (SA4 and SA5 yellow, SA6, SA7 and SA8 white) selected in 1995.

Populations	**Acid tolerant**		**Acid Susceptible**		**Total**
	S_4	**S_6**	**S_4**	**S_6**	
SA-4	0	1	0	3	4
SA-5	0	3	0	1	4
SA-6	1	0	1	0	2
SA-7	0	0	1	0	1
SA-8	1	0	0	0	1
Total	2	4	2	4	12

Table 2. Crosses, pedigree, acid soil reaction, and seed availability of twelve selected maize lines.

Cross	Pedigree	Reaction [1/]	Available F_2 [2/]	No. Line
1	SA6-C2HC(6x1)-3-2-7-6	T		1
	SA6-C2HC(3x11)-4-1-1-6	S		2
2	SA6-C2HC(6x1)-3-2-7-6	T	1	1
	SA7-C2HC(3x5)-2-1-1-10	S		3
3	SA7-C2HC(3x5)-2-1-1-10	S		3
	SA8-C1HC(27x3)-1-3-6-8	T		4
4	SA4-HC7-1-4-1-1-1-1	T	2	5
	SA5-HC1-3-8-3-2-6-4	S		6
5	**SA5-HC1-1-5-1-1-1-7**	**T**	**3**	**7**
	SA4-HC7-1-4-1-2-11-1	**S**		**8**
6	SA5-HC1-3-9-3-2-4-6	T	4	9
	SA4-HC7-1-4-5-3-3-10	S		10
7	SA5-HC1-3-9-3-2-5-4	T	5	11
	SA4-HC7-1-4-5-3-8-3	S		12

[1/] Reaction: T= tolerant, S= susceptible.

[2/] $F_{1:2}$ generations of five crosses available.

Table 3. Evaluation "*per se*" of twelve maize lines for tolerance to acid soils in three environments: Normal-fertile soil (Palmira) and two in acid soil (Matazul and Villavicencio 1), Colombia, 1997.

Line	**Plant Vigor (1-5)**† **Matazul**	**Yield (1-5)**† **Villavo1**	**Yield (1-5)**† **Palmira**	**Yield (t ha^{-1}) Villavo1**	**Yield (t ha^{-1}) Palmira**
1	2.0	2.5	1.5	1.5	3.4
2	4.0	3.0	2.0	1.6	3.3
3	1.5	3.5	3.5	0.7	1.9
4	2.5	2.5	3.0	1.1	2.6
5	1.5	3.5	2.5	0.5	2.3
6	2.5	2.5	2.5	1.3	2.5
7	**1.5**	**2.0**	**3.0**	**1.3**	**2.4**
8	**4.5**	**3.5**	**3.0**	**0.6**	**2.0**
9	1.0	2.0	2.5	0.8	2.8
10	3.5	5.0	4.0	0.2	1.4
11	1.0	2.0	2.0	1.6	2.4
12	4.0	4.5	4.0	0.2	1.1

† Visual judgment, 1 = Good yield, 5= Poor yield.

Based on the results shown in the Table 3, we used line 7 tolerant x line 8 susceptible (SA5-HC1-1-5-1-1-1-7 x SA4-HC7-1-4-1-2-11-1= cross 5) as parental lines in this study (Table 2). The SA4 and SA5 source populations are experimental cultivars under improvement by full-sib family selection method for tolerance to soil acidity.

Population SA-4 has yellow-dent kernels and was developed from Mezcla Amarilla, Amarillo Cristalino-1, Amarillo Dentado, SA-3, Cogollero, and Suwan-La Posta germplasms from CIMMYT, CMS-30 from Brazil and MB 123 from Colombia. SA-4 is heterotic with the populations SA-3 and SA-5 (Pandey *et al*., 1994, Pandey *et al*., 1995). Population SA-5 has yellow-flint kernels and was developed from Mezcla Amarilla, SA-3, Amarillo Dentado, Amarillo Cristalino, Cogollero, and Amarillo Cristalino-2 germplasms from CIMMYT and CMS-36 from Brazil. SA-5 is heterotic with the population SA-4 (Pandey *et al*., 1994, Pandey *et al*., 1995).

In the acid soils, the yield of line 7 (tolerant) was significantly greater (twice or more) than the yield of the line 8 (susceptible) 1.3 t ha^{-1} vs. 0.6 t ha^{-1}. In the normal–fertile soil (Palmira), the yield of line 7 was similar to line 8 (Table 3).

In 1997B, second cycle, F_2 seed of cross 5 was planted (three ears). Two ears were planted in Palmira (PAL) and one in Santander de Quilichao (SQ). In PAL and SQ, the seed from each ear was planted in 150 hills (two seeds per hill, then thinned to one plant per hill). The 150 plants, from each ear, were tagged. In PAL, leaf samples were harvested from 221 F_2 plants before pollination from the leaf nearest to the ear, quick frozen in liquid nitrogen, freeze dried, and stored at -20° C until delivery at Applied Molecular Genetics Laboratory (ABC) CIMMYT, Int., El Batán, Mexico. The $F_{2:3}$ lines were produced by self-pollinating F_2 plants. At harvest, 214 families were selected that had enough seeds for field trials (the families 11, 36, 58, 91, 104,1 27, and 160 were declared missing).

Field trials

In 1998B, second cycle, the two parents (P7 = line 7 and P8= line 8), the F_1, and the 214 $F_{2:3}$ progenies were planted. The F1, P7, and P8 were repeated twice as entries in each replication. The trials were grown in four locations in Colombia: International Center for Tropical Agriculture (CIAT) in Palmira (PAL), Colombian Corporation of Agricultural Research (CORPOICA) research station, La Libertad in Villavicencio (V), CORPOICA research station, Turipaná in Cereté (TUR) and “Nueva Esperanza” farm in Ayapel (AYA). These locations included six environments: PAL and TUR are normal-fertile soils, V55 and V65 are acid soils with about 55 and 65% of aluminum saturation in Villavicencio and AYA65 and AYA80 are acid soils with 65 and 80% of aluminum saturation in Ayapel (Table 4).

The planting and harvest dates of experiments shown in Table 4 were as follows: V55, September 23/98 - January 26/99; V65, September 24/98 – January 27/99; PAL, September 10/98 – January 21/99; TUR, September 23/98 – February 1/99; AYA 80, September 28/98 – February 2/99; AY65, October 30/98 – February 17/99.

Experiments were evaluated in an alpha lattice design (22x10) with two replications for each environment. In PAL, V55, and V65 the plot size was a single row, 2.5 m long, 0.75 m between rows, and one plant per hill spaced 0.25m within the row. In TUR, AYA65, and AYA80 the plot size was a single row, 2.5 m long, 0.80 m between rows, and one plant per hill spaced 0.20 m within the row.

Table 4. Geographical location and soil's characteristics of the environments that were used for the evaluation trials at acid and non-acid soils in Colombia during 1998B growing season.

Environment	Latitude	Longitude	Altitude	pH	P	Al saturation.
	Degrees		m		ppm	%
Villavicencio (V55)[1/]	4° 06' N	73° 29' W	400	4.8	9.4	62.1
Villavicencio (V65)[1/]	4° 06' N	73° 29' W	400	4.8	10.6	68.3
Ayapel (AYA65)[1/]	8° 18' N	75° 08' W	22	4.8	1.8	65
Ayapel (AYA80)[1/]	8° 18' N	75° 08' W	22	4.4	1.0	81
Palmira (PAL)[2/]	3° 30' N	76° 19' W	965	7.1	90.9	< 1
Cereté (TUR)[2/]	8° 51' N	75° 49' W	13	6.5	20	< 1

[1/] Acid - soil

[2/] Non-acid soil

Plots were over-planted with two seeds per hill and thinned to one plant per hill. Planting and harvesting were done by hand. Weeds and insects were controlled as needed. Female flowering (FEM) was measured as the number of days from sowing to 50% of the plants were silking. Male flowering (MAS) was recorded as the days to 50% of the plants were in anthesis. Anthesis-silking- interval (ASI) was calculated as the difference between the FEM and MAS family means. Grain yield (YLD) in t ha^{-1} was calculated as 80% of the ear weight adjusted to 150 g kg^{-1} moisture. The number of ears per plant (PROLI) was calculated by dividing the total number of ears harvested by the total number of plants in the

plot. Plant and ear height (PH and EH) were measured as the number of cm from the soil surface to the lowest tassel branch or the leaf nearest to the ear, respectively.

Data analysis

Adjusted means and genotypic variances per trial were calculated per family for each trait and environment using the PROC MIXED procedure in SAS (SAS Institute, 1988). Simple Pearson correlation coefficients were calculated among traits within and combined across environments. Broad-sense heritabilities (**h^2**), on an $F_{2:3}$ progeny mean basis, were estimated, according to Hallauer and Miranda (1988), as follows:

$$h^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \frac{\hat{\sigma}^2_{ge}}{e} + \frac{\hat{\sigma}^2}{re}} ,$$

Where: $\hat{\sigma}^2_g$ =genotypic variance, $\hat{\sigma}^2_{ge}$ =genotype by environment interaction variance, $\hat{\sigma}^2$ =error variance, **e** =number of environments, and **r**= replications.

SSR analysis

From the two parental lines (P7 and P8) and 221 $F_{1:2}$ plants, maize genomic DNAs were isolated, quantified, suspended, and stored. The genomic DNA isolation protocol is based on the method of Saghai-Maroof *et al*., (1984) and the details of SSR protocols are given in Hoisington *et al*., (1994). Basically, the genomic DNA isolation method included 400 mg of ground, lyophilized tissue sample with 9.0 ml of warm CTAB (Mixed alkyltrimethyl-ammonium bromide, Sigma ® M-7635) extraction buffer added. Tubes were incubated for 90 minutes with continuous gentle rocking in a 65° C oven. After the tubes

cooled down, 4.5-ml chloroform/octanol (24:1) were added and mixed very gently. Samples were centrifuged for 10 minutes at 2700 rpm. After samples were centrifuged, the top aqueous was pipette off and 30 µl of RNase A and 6.0 ml isopropanol were added. Precipitated DNA was removed, washed, and suspended in TE (Tris-EDTA buffer).

The Ultra Violet (UV) quantification of DNA for each sample was done in the Beckman DU-65 ® Spectrophotometer. After UV quantification the concentration was adjusted for each DNA sample to 0.3 µg/µl with TE and stored at 4° C.

DNA digestibility test was done (Hoisington *et al*., 1994). The results showed that the DNA was degraded in almost all samples. Unlike for RFLPs or AFLPs, the quality of the template DNA is less critical for SSRs. For this reason, SSR (PCR) technology was used instead of RFLP technology. The PCR process (amplification) started with the preparation of a bulk reaction mix containing all the components listed in Table 5.

DNA samples and bulk mix reactions were placed into labeled tubes, and samples were overlaid with one drop of ultrapure mineral oil. The tubes were placed in the PCR machine and the standard program was run (Table 6). After amplification was done, 3 to 4 µl of 5X SGB (Sample Gel Buffer) were added to each tube. Twenty µl of each sample were loaded in a 3% agarose gel (1.5% Metaphor ® FMC, Rockland, NY and 1.5% Seakem agarose ®) prepared with 1X TBE (Tris-borate EDTA). The gels were placed in electrophoresis device into 1X TBE at 80 V, constant voltage for about 2 to 3 hours. After electrophoreses, the gels were stained in 1 µg/ml ethidium bromide (100 µl of 10 mg/ml ethidium bromide in 1000 ml d H_2O) for 10 minutes with gentle shaking. The gels were rinsed in d H_2O for 20 minutes and placed onto an UV transilluminator and photographed.

Table 5. Final and reaction volumes used of each component in PCR process for SSR analysis, based on the procedures described by Hoisington *et al*., (1994).

STOCK	**[FINAL]**	**20 µl RXN**
ddH2O Sigma Cell Culture Water ®	-	3.6
Taq Buffer (10X; Mg-free)	1X	2.0
$MgCl_2$ (50 mM)[2]	2.5 mM	1.0
dNTP Mix (2.5 mM each)	150 µM each	1.2
Taq Enzyme (5 U/µl)	1U	0.2
Glycerol (100%)	10%	2.0
Primers, F + R (1.0 µM each)	0.25 µM each	5.0
DNA (10 ng / µl)	50 ng	5.0

About 700 SSR primers were used to screen the two parental lines (P7 and P8). The primers basically came from University of Missouri, Columbia or Applied Molecular Genetics Laboratory (ABC) CIMMYT, Int., El Batán, Mexico. Those primers are in the public domain and they are referenced in MaizeDB, 2000. Only those primers that showed good and clear polymorphism were chosen and then they were amplified in the entire population (221 individuals).

Table 6. The Standard PCR program optimized for *ERICOMP* TwinBlock™/MJ Research DNA Engine Tetrad™ System Thermocyclers, based on procedures described by Hoisington *et al*., (1994).

1 cycle of:	**30 Cycles of:**	**1 cycle of:**
93 °C for 1 minute	93 °C for 1 minute	72 °C for 5minutes
	X °C for 2 minutes [1]	
	72 °C for 2minutes	

[1/] X ranged between 50 and 68° C. For most SSR primers the annealing temperature was 60 ° C. Other temperatures used were 50, 52, 54, 56, 58 ° C.

Linkage map and QTL determination

The program HyperMapData–version 1.6 (software developed at CIMMYT, Int., Mexico) was used for the entry, verification (two readers), and preliminary statistical analysis. One file was conformed to molecular genotypic data of the SSR photographs were obtained from the protocols described above. Each marker locus was tested by a chi-square goodness of fit test for the expected Mendelian segregation ratio, 1:2:1 for co-dominant loci and 3:1 for dominant loci. HyperMapData software created, in special format, a new file (called "export") to be used by MapMaker/EXP 3.0 (Lander *et al*., 1987). The best SSR polymorphic primers (118) were chosen to construct the linkage map of the $F_{1:2}$ population.

The linkage map was constructed using the computer program MapMaker/EXP 3.0. This program is in the public domain and it could be accessed on line (see references). The basic procedure used to construct the map included the follow steps:

1) The exported file from HyperMapData was loaded in MapMaker/EXP using the "prepare" command.

2) All loci were included using the command “sequence” in order to start the linkage analysis. We began this session by performing a classical “two-point” analysis. The “group” command was used, instructing the program to divide the markers into linkage groups. MapMaker calculates the maximum-likelihood distance and corresponding LOD score between the two markers. Two markers were considered linked if the LOD > 3.0 and if the distance < 80 Haldane cM (by default). Also MapMaker considers linkage transitive, that is, if marker A is linked to B and B is linked to C, then A is linked to C.
3) Once the suspected linkage groups were identified, we examined all three-point crosses per each linkage group. The most likely order of the three must have a relative log-likelihood of 0.0, while the others have negative values. Other commands used were “compare” and “try”. The “compare” command computes the maximum likelihood map for each specified order of N markers (N!/2 possible orders) and it reported the 20 most likely ones. The “try” command tries to place specific marker in each interval in the framework.
4) Finally, using the “ripple” command the map order was verified. The “ripple” command instructs MapMaker to permute the order of neighboring markers, and to compare the likelihoods of the resulting maps.

QTL detection was performed with CIM (Composite Interval Mapping), a software program developed at CIMMYT (Jiang, 1998). This program, in FORTRAN language is based on four models, which should maximize the QTL detection and to minimize the risk of including false QTL. The models are defined by the family type F_2, F_3 or F_7 (Recombinant Inbred Lines), the minimum distance of a marker used as cofactor away from the testing interval (window size), additive effect only or both additive and dominance (for F_2

population), the number of traits to be jointly analyzed, and number and position of selected markers to be used as cofactors. The models are describe as follows:

Model 1: Simple Interval Mapping (SIM). This model was used only for selecting markers as cofactors. It corresponds to SIM (Lander and Botstein, 1989) and Model III of Zeng (1994). "Whenever a likelihood ratio (LR) value exceeds the threshold (critical values selected), the nearest marker is then selected" (Jiang, 1998 p 9). The chromosome (s) number (s) that contained selected marker (s), number of marker (s) from each chromosome, and marker number was (were) written into the model 2.

Model 2: CIM with only unlinked markers as cofactors (Model II of Zeng, 1994). The basis for this model is "if selected marker is on the chromosome under testing, this marker will be removed from the list of cofactors until the analysis moves to the next chromosome. This model gives a smaller residual variance and is supposed to have the highest power for QTL detection in absence of multiple linked QTLs" (Jiang, 1998 p 9). If some new cofactors were found, they were added at the Model 3.

Model 3: CIM with the selected markers as cofactors and two markers flanking the interval under testing but at least 30 cM away from the interval (Model I of Zeng, 1994). "The purpose of using flanking markers as cofactors is to block the effects from possible QTLs in neighboring intervals" (Jiang, 1998 p 9).

Model 4: Basically the same as Model 3, but with 20 cM as the minimum distance.

One QTL was declared when a LR value either equaled or passed the critical value under any model. The "ghost" QTLs were identified when they were suggested by Model (1) and/or (2) but later disappeared under Model (3) and/or (4) (Jiang, 1998). The critical values mentioned above must not be too high to miss any true QTL and must not be too low to

declare false QTLs. The general relationship between LOD and LR is: LR=2 ln (10) LOD. The LOD score (Z) is defined as the base-10 log likelihood ratio test statistic. In practical linkage analysis, LOD=3.0 means that linkage at $\theta = \hat{\theta}$ is 1,000 times more likely than at $\theta = 0.5$. Jiang (1998) presents a SAS program to calculate the Type I error (α level) corresponding at different LOD and LR scores. In the Tables 7 and 8 critical values used in a single and combined traits analysis in this study are presented.

QTLs were declared by the Model 4 (window = 20 cM), LOD=2.5 with its respective LR critical values (Table 7); i.e., for each trait in a single environment the LR critical value was 11.51; for each trait in three acid soil combined environments (COM. AS), the LR critical value was 18.68, and for each trait in two normal-fertile combined environments (COM.NS) the LR critical value was 15.27. The chromosome and estimated position in centi-Morgans were recorded and QTLs effects were obtained from the output file QTLest of the CIM (Composite Interval Mapping) software.

Table 7. Critical values for QTL analysis for multiple environments or traits on F_2 maize population (df=3), as reported by Jiang (1998).

Number of traits	**LR (df) LOD=3.0 / α = 0.00317**	**LR LOD=2.5 / α = 0.00925**
1	13.81 (3)	11.51 (3)
2	17.83 (5)	15.27 (5)
3	21.44 (7)	18.68 (7)

Table 8. Critical values for testing QTL by environment interaction on F_2 maize population, as reported by Jiang (1998).

Number of traits	LR (df) α=0.05
2	5.99 (2)
3	9.49 (4)
4	12.59 (6)

Jiang (1998) defines QTLs effects as " The negative estimates of additive effects (add.) mean that the substitution effect of "A" allele from parent 1 for allele "B" from parent 2 tends to reduce the trait at this locus. Negative dominance effects (dom.) means that the mean of heterozygous is less than the mean of two homozygous at this locus"(p 14).

The phenotypic variance explained (R^2 values) by the detected QTL were obtained using QTLR2 application of the CIM software (Jiang, 1998). The interpretation and restrictions given by Jiang, (1998) about R^2 values are: " The non-random segregation among QTL exists due to linkage or just by chance, the R^2 for each QTL will not sum up to the R^2 value from the multiple regression. In addition, the nature of the method used for QTL detection is to search for individual QTL (chromosome segments) with significant association with the trait under analysis. Some QTL and the estimated QTL effect would be partially due to random variation only. The QTL effects are usually overestimated. Therefore, the summation from the individual QTL R^2 will be greater than the total QTL R^2 value. But the possibility of the total QTL R^2 value greater than the sum of individual QTL R^2 exists though it may be rare"(p 20).

The LR scores for QTL by environment interaction (QxE) for combined across environments (COM. AS and COM. NS) were obtained from the output file QTLest of the CIM (Composite Interval Mapping) software at each respective position. The methodology to declare significant interaction was described above to declare a QTL. The critical values are presented in the Table 7. Wherever a LR value reached the critical value, the QxE was declared significant (α=0.05) and the QTL was defined as unstable. The LR critical values used were, COM. SA (9.49) and COM. NS (5.99), for three and two environments, respectively.

RESULTS AND DISCUSSION

The Ayapel environment with 80% of Al saturation (AYA80 experiment) was declared missing. One possible explanation was the higher Al saturation that was deeper than 30 cm (>90% Al Sat.). The grain yield for all entries was zero. Hence, five environments were considered for all analysis.

The relative efficiency of the alpha lattice design (data not shown) was at least equal to randomize complete block design (RCBD). The efficiency was zero for ASI in all environments except PAL, and for PROLI in TUR. For remaining traits and environments, relative efficiency ranged from 3% (MAS in PAL) to 50% (MAS in AYA65). Yield values for efficiency were 13, 26, 1, 30 and 22% in V55, PAL, V65, TUR, and AYA65, respectively. For this reason, adjusted means were used in all analysis.

Adjusted means, standard errors (SE), genetic variance entry mean basis (Gen.Var.), and broad-sense heritabilities (h^2) for parental lines and F_3 families for each of the traits at the four locations and the five environments evaluated are presented in Tables 9 to 15.

Under acid soil environments, yield showed transgressive segregation (Table 12). The range among F_3 families increased with Al saturation level (V55 to V65). Also, the F_3 families means decreased with Al saturation level (V55, V65, and AYA65).

Yields of F_3 families means were 0.9, 0.8, and 0.4 t ha^{-1} at V55, V65, and AYA65, respectively. These yields are only 20, 18 and 9% of best normal-fertile soil yield in PAL (4.5 t ha^{-1}). Except at V55, the tolerant parental line (P7) yielded more at V65 (64%) and at AYA65 (60%) than the susceptible parental line (P8).

Table 9. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for female flowering (FEM) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	**Environments**[1/]				
	V55	**PAL**	**V65**	**TUR**	**AYA65**
Mean (P7)	60.2 ± 0.8	62.0 ± 0.6	58.8 ± 1.2	59.0 ± 0.8	63.2 ± 1.8
Mean (P8)	60.7 ± 0.8	59.7 ± 0.6	61.4 ± 1.2	56.5 ± 0.8	60.0 ± 1.8
Mean (F_3)	60.0 ± 1.3	59.1 ± 1.7	60.8 ± 1.4	56.3 ± 1.4	60.5 ± 3.4
Range (F_3)	56.5 / 64.1	54.6 / 63.1	57.1 / 64.4	52.4 / 61.6	52.6 / 70.2
Gen. Var.	0.24	2.25	0	1.29	5.47
h^2	0.16	0.78	NE [2/]	0.68	0.52

[1/] Environments were Villavicencio (V55 with 55% Al saturation and V65 with 65% Al saturation), Palmira (PAL), Turipaná in Cereté (TUR), and Ayapel (AYA 65 with 65% Al saturation).

[2/] Heritability was not estimable because estimate of genetic variance was zero

Except for FEM, MAS, ASI at AYA65, acid soil environments tended to reduce the genetic variance of all traits. According to Ribaut et al., (1997) the reduction of genetic variance for drought stress could reduce the power of QTL detection. Additionally, other studies have shown that stressed environments cause genetic variance reductions (Veldboom and Lee, 1996). Genetic variance was zero for FEM at V65, MAS at V65, ASI at V55, V65, and EH at V55, and V65. Estimates of zero genetic variance also did not permit the estimation of heritabilities. The estimates of heritabilities in acid environments (V55, V65 and AYA65) were generally lower for all traits but yield in V65 (36%) and in AYA65 (60%),

Table 10. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for male flowering (MAS) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	**Environments**[1/]				
	V55	**PAL**	**V65**	**TUR**	**AYA65**
Mean (P7)	58.6± 0.8	62.0 ± 0.6	58.2 ± 1.1	57.5 ± 1.0	62.7 ± 1.7
Mean (P8)	59.4 ± 0.8	58.6 ± 0.6	60.1 ± 1.1	56.2 ± 1.0	56.8 ± 1.6
Mean (F_3)	58.7 ± 1.3	59.0 ± 1.6	59.9 ± 1.3	55.4 ± 1.6	58.1 ± 2.7
Range (F_3)	53.4 / 62.8	55.0 / 63.0	56.9 / 63.0	51.2 / 62.5	52.6 / 67.0
Gen. Var.	0.42	1.81	0	1.70	2.80
h^2	0.27	0.70	NE [2/]	0.65	0.40

[1/] See Table 9.

[2/] See Table 9.

which are similar to the range of earlier reports (38%) from CIMMYT (Duque-Vargas *et al.*, 1994; Pandey *et al.*, 1994; Borrero *et al.*, 1995). The estimates of heritability are lower than the values reported for intermediate (61%) and severe drought stress (74%) by Ribaut *et al.*, (1997).

Broad-sense heritabilities (h^2) for acid soil (COM. AS), normal soil (COM. NS) and combined across environments (COM. ALL) are presented in Table 16. Heritability estimates for acid soil combined were very low or near zero values. One possible explanation for this is the very low or zero estimates of genetic variance in acid soil environments. V55 and V65 environments were in the same location and were planted almost at the same time. The objective of the V55 and V65 environments was to compare

Table 11. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for anthesis-silking-interval (ASI) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	Environments[1/]				
	V55	PAL	V65	TUR	AYA65
Mean (P7)	1.5± 0.6	0.096 ± 0.5	0.8 ± 0.5	1.8 ± 0.6	1.3 ± 1.1
Mean (P8)	1.3 ± 0.6	0.94 ± 0.5	1.3± 1.5	0.5 ± 0.6	3.3 ± 1.1
Mean (F_3)	1.3 ± 0.8	0.04 ± 1.0	0.9 ± 0.7	0.8 ± 1.1	2.4 ± 2.3
Range (F_3)	0 / 6.0	-5.2 / 2.9	-2.5 / 3.0	-4.0 / 5.5	-3.0 / 12.0
Gen. Var.	0	0.27	0	0.49	2.61
h^2	NE [2/]	0.31	NE [2/]	0.43	0.51

[1/] See Table 9.

[2/] See Table 9.

Table 12. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for yield (YLD) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	Environments[1/]				
	V55	PAL	V65	TUR	AYA65
Mean (P7)	0.5± 0.2	2.6 ± 0.4	1.1 ± 0.2	1.6 ± 0.4	0.1 ± 0.1
Mean (P8)	0.9± 0.2	2.7 ± 0.5	0.7 ± 0.2	0.6 ± 0.4	0.06 ± 0.1
Mean (F_3)	0.9 ± 0.3	4.5 ± 1.1	0.8 ± 0.4	2.0 ± 0.7	0.4 ± 0.2
Range (F_3)	0.2 / 1.7	1.7 / 7.6	0.07 / 2.7	0.5 / 4.7	0 / 1.3
Gen. Var.	0.0009	0.94	0.046	0.43	0.04
h^2	0.01	0.72	0.36	0.70	0.60

[1/] See Table 9.

Table 13. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for ears per plant (PROLI) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	Environments[1/]				
	V55	PAL	V65	TUR	AYA65
Mean (P7)	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.3 ± 0.1
Mean (P8)	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.2 ± 0.1
Mean (F_3)	0.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	0.4 ± 0.2
Range (F_3)	0.3 / 1.1	0.6 / 1.7	0.3 / 1.0	0.4 / 1.4	0 / 1.0
Gen. Var.	0.0008	0.01	0.005	0.016	0.02
h^2	0.06	0.49	0.29	0.54	0.57

[1/] See Table 9.

Table 14. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for plant height (PH) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	Environments[1/]				
	V55	PAL	V65	TUR	AYA65
Mean (P7)	122 ± 6	205 ± 7	130 ± 6	187 ± 10	163 ± 9
Mean (P8)	123 ± 6	156 ± 7	133 ± 6	109 ± 10	104 ± 9
Mean (F_3)	126 ± 8	191 ± 14	127 ± 8	165 ± 13	137 ± 14
Range (F_3)	96 / 144	140 / 246	103 / 152	119/ 193	107 / 170
Gen. Var.	1.78	119.22	6.77	130.41	74.25
h^2	0.03	0.62	0.12	0.74	0.39

[1/] See Table 9

Table 15. Adjusted means ± SE of parental lines and F_3 maize families, genetic variance (Gen. Var.), and broad-sense heritabilities (h^2) for ear height (EH) in four locations and five environments evaluated in Colombia, 1998B.

ITEM	Environments[1/]				
	V55	PAL	V65	TUR	AYA65
Mean (P7)	47 ± 3	99 ± 5	42 ± 4	71 ± 8	61 ± 6
Mean (P8)	43 ± 3	74 ± 5	47 ± 4	45 ± 8	32 ± 5
Mean (F_3)	45 ± 4	89 ± 10	44 ± 5	68 ± 8	50 ± 8
Range (F_3)	34 / 59	64 / 143	29 / 58	44 / 89	31 / 73
Gen. Var.	0	41.06	0	32.79	16.93
h^2	NE[2/]	0.45	NE[2/]	0.51	0.29

[1/] See Table 9.

[2/] See Table 9.

two levels of Al saturation, but the results were not consistent with the stress level (Table 16). Probably V55 did not produce enough stress to discriminate within the segregation population or external factors affected differentially both environments. Also parent 7 (tolerant) yielded less that susceptible parent 8 at V55 (Table 12). Ayapel (AYA65) with 65% of Al saturation is another acid location, and it possibly could have other soil components very different from those at V65. Normal-fertile soils environments were similar to those reported in the literature although, in some cases, slightly lower.

The heritability (h^2) of ASI could not be estimated across environments. In the single acid environments only AYA65 presented a value of 0.51 that was greater than for the

Table 16. Estimates of broad-sense heritabilities (h^2) in maize for acid soil (COM. AS), normal soil (COM. NS), and combined across environments (COM. ALL). Colombia, 1998B.

TRAIT	COM.AS	COM. NS	COM. ALL
FEM	0.05	0.72	0.48
MAS	0.05	0.66	0.47
ASI	NE[1/]	NE[1/]	NE[1/]
YLD	NE[1/]	0.62	0.46
PROLI	NE[1/]	0.45	0.32
PH	0.02	0.67	0.52
EH	0.02	0.56	0.41

[1/] Heritabilities were not estimable because estimates of genetic variance were zero.

normal soils (0.31 in PAL and 0.43 in TUR). Except for ASI being important to drought stress, there was no evidence to consider ASI important for acid soil tolerance (Ribaut *et al.*, 1996). One possible explanation could be inaccuracy on data measurement. Ribaut *et al.*, 1996 suggest that high accuracy of the data measured on an individual plant basis could improve estimates of heritability especially under stress conditions. In this study all traits were measured on a plot basis. In future work, data must be taken on an individual plant basis with more accuracy and appropriate sample size.

Phenotypic linear correlations were calculated between traits in each environment, and the results are presented in Tables 17 to 21. FEM and MAS were the largest highly correlated values (>0.73 and $p\leq0.01$) under all environments. ASI was highly correlated in

all environments with FEM (positively). Also, ASI was highly correlated with MAS (negatively) in all environments, except AYA65.These results for flowering traits are similar to the results reported for drought stress by Ribaut *et al*., (1996). The results of this study add more evidence that stress conditions, such as drought, causes major delays in silk emergence and has less effect on MAS.

For yield, although the Pearson's coefficient values were not high, they were highly significant ($p \leq 0.01$). Across locations yield was negatively correlated and consistent with FEM, MAS, and ASI except with ASI in TUR. Also yield had positive highly significant correlation with PROLI in all environments ranging from 0.47 at PAL to 0.74 at AYA65.

Table 17. Phenotypic linear correlation (Pearson's coefficient) between maize traits at Villavicencio with 55% Al saturation (V55), Colombia, 1998B.

	TRAITS[1/]					
TRAITS[1/]	**MAS**	**ASI**	**YLD**	**PROLI**	**PH**	**EH**
FEM	0.82**	0.31**	-0.39**	-0.26**	-0.23**	-0.19**
MAS		-0.28**	-0.24**	-0.13*	-0.11	-0.05
ASI			-0.27**	-0.21**	-0.21**	-0.23**
YLD				0.66**	0.30**	0.33**
PROLI					0.17*	0.22**
PH						0.77**

[1/] Traits include number of days from planting to female (FEM) and male (MAS) flowering, anthesis-silking-interval (ASI), yield (YLD), number of ears per plant (PROLI), and plant (PH) and ear (EH) height.

** , * significant at the < 0.01 and < 0.05 probability level, respectively.

Table 18. Phenotypic linear correlation (Pearson's coefficient) between maize traits at Palmira (PAL), Colombia, 1998B.

	TRAITS[1/]					
TRAITS[1/]	**MAS**	**ASI**	**YLD**	**PROLI**	**PH**	**EH**
FEM	0.82**	0.38**	-0.26**	-0.06	0.15	0.04
MAS		-0.21**	-0.26**	0.007	0.24**	0.16*
ASI			0.04	-0.09	-0.15*	-0.19**
YLD				0.47**	0.13	0.05
PROLI					0.20**	0.26**
PH						0.55**

[1/] See Table 17.

Table 19. Phenotypic linear correlation (Pearson's coefficient) between maize traits at Villavicencio 65% Al Saturation (V65), Colombia, 1998B.

	TRAITS[1/]					
TRAITS[1/]	**MAS**	**ASI**	**YLD**	**PROLI**	**PH**	**EH**
FEM	0.85**	0.38**	-0.40**	-0.35**	-0.26**	-0.19**
MAS		-0.15*	-0.28**	-0.23**	-0.15*	-0.09
ASI			-0.24**	-0.26**	-0.22**	-0.19**
YLD				0.60**	0.41**	0.36**
PROLI					0.36*	0.30**
PH						0.75**

[1/] See Table 17.

** , * significant at the < 0.01 and < 0.05 probability level, respectively.

Table 20. Phenotypic linear correlation (Pearson's coefficient) between maize traits at Turipaná (TUR), Colombia, 1998B.

	TRAITS[1/]					
TRAITS[1/]	**MAS**	**ASI**	**YLD**	**PROLI**	**PH**	**EH**
FEM	0.73**	0.17**	-0.30**	-0.33**	0.13	-0.01
MAS		-0.54**	-0.36**	-0.34**	0.04	-0.10
ASI			0.15*	0.09	0.09	0.13
YLD				0.51**	0.25**	0.18**
PROLI					0.24**	0.25**
PH						0.59**

[1/] See Table 17.

Table 21. Phenotypic linear correlation (Pearson's coefficient) between maize traits at Ayapel 65% Al Saturation (AYA65), Colombia, 1998B.

	TRAITS[1/]					
TRAITS[1/]	**MAS**	**ASI**	**YLD**	**PROLI**	**PH**	**EH**
FEM	0.73**	0.59**	-0.63**	-0.65**	-0.32**	-0.13
MAS		-0.10	-0.47**	-0.43*	-0.28**	-0.10
ASI			-0.36**	-0.45**	-0.13	-0.08
YLD				0.74**	0.38**	0.19**
PROLI					0.37*	0.21**
PH						0.63**

[1/] See Table 17.

** , * significant at the < 0.01 and < 0.05 probability level, respectively.

This correlations were lower than previous reports from CIMMYT of 0.84** (Duque-Vargas *et al*., 1994; Pandey *et al*., 1994; Borrero *et al*., 1995). Except in PAL yield correlated positive and highly significant in all environments with PH and EH.

The SSR linkage map for F_2 segregating population is presented in Figure 1. The final values included were 221 individuals and 118 SSR primers. Primers included were 95 codominant and 23 dominant/recessive. The total length of the map was 1836.2 cM with a mean density of 15.56 cM. Between the primers bmc 1209 and bngl 619 on the chromosome 9, was located the major gap with 61.6 cM

The map was compared with the Maize DataBase (MaizeDB, 2000) to obtain evidence about linkage groups and position of SSR primers in each bin. The marker bmc 1091 was mapped in a new position on chromosome 1, at 145 cM, instead the current position in chromosome 9 (bin 9.05-9.06). The marker bmc 1019 was mapped in a new position in chromosome 3, at 34.9 cM, instead the current position on chromosome 4 (at bnlg 1019a probed site). The marker umc 1087 was mapped in a new position on chromosome 3, at 37.7 cM, instead the current position on chromosome 6 (bin 6.05). The marker dup 7 with unknown position in MaizeDB was mapped on chromosome 5 at 90.1 cM. There were no other significant inconsistencies between the map in Figure 1 and the MaizeDB.

The results of the QTL analysis are presented in Tables 22 to 28. The structure of presentation was done per trait, for each environment, and combined across environments (acid soil, COM.AS; and normal-fertile soil, COM. NS). Chromosome, QTL position in centi-Morgans, nearest SSR locus, LR score, additive and dominant effects (add. and dom.), and R^2 (phenotypic variance explained) were included.

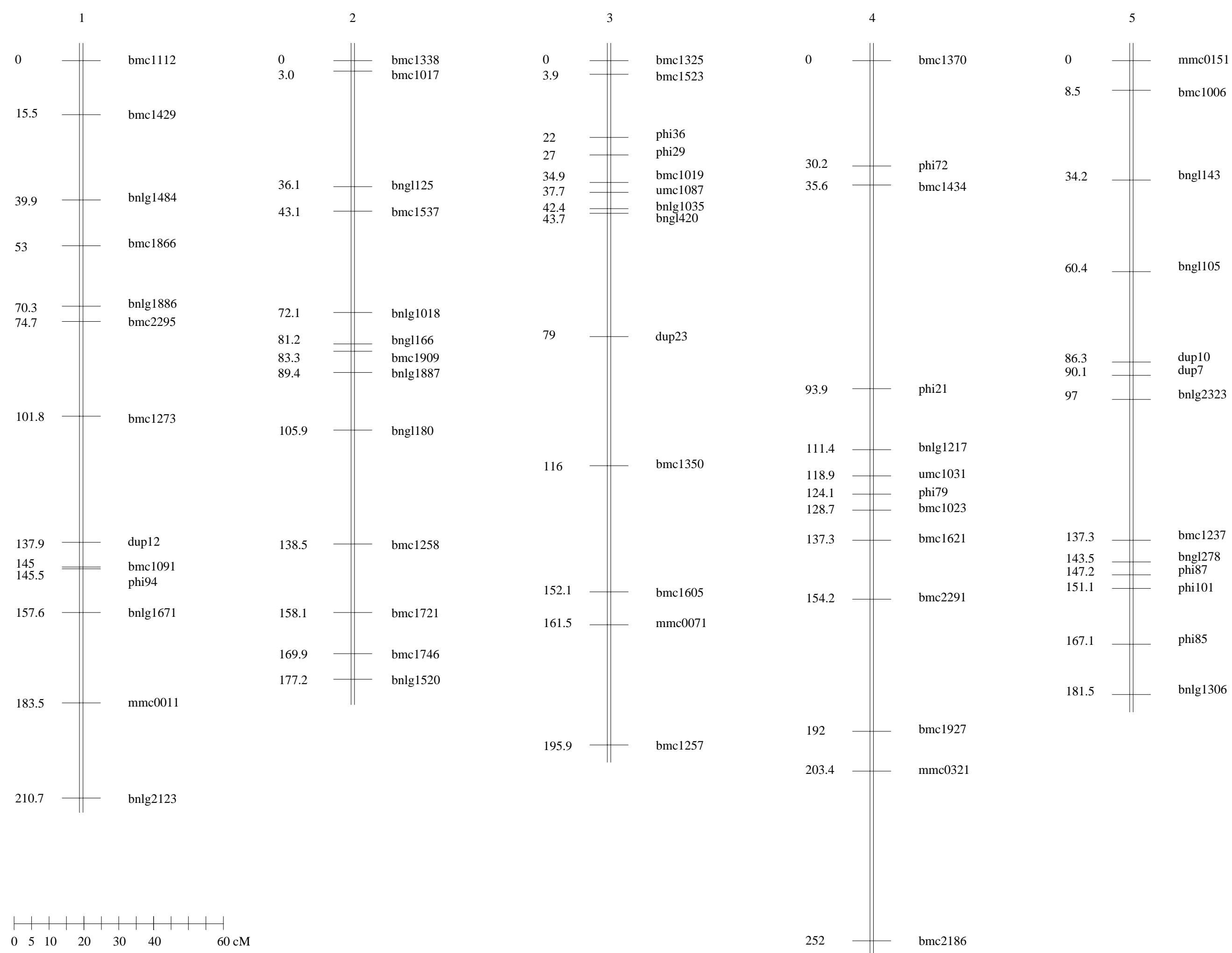

Figure 1. Linkage map of maize F_2 segregating population from the cross SA5-HC1 X SA4-HC7 developed with 118 SSRs primers in 221 F_2 plants. Chromosome number is on the top, SSR primer names are on the right, and cumulative distances in centi-Morgan (cM) on the left.

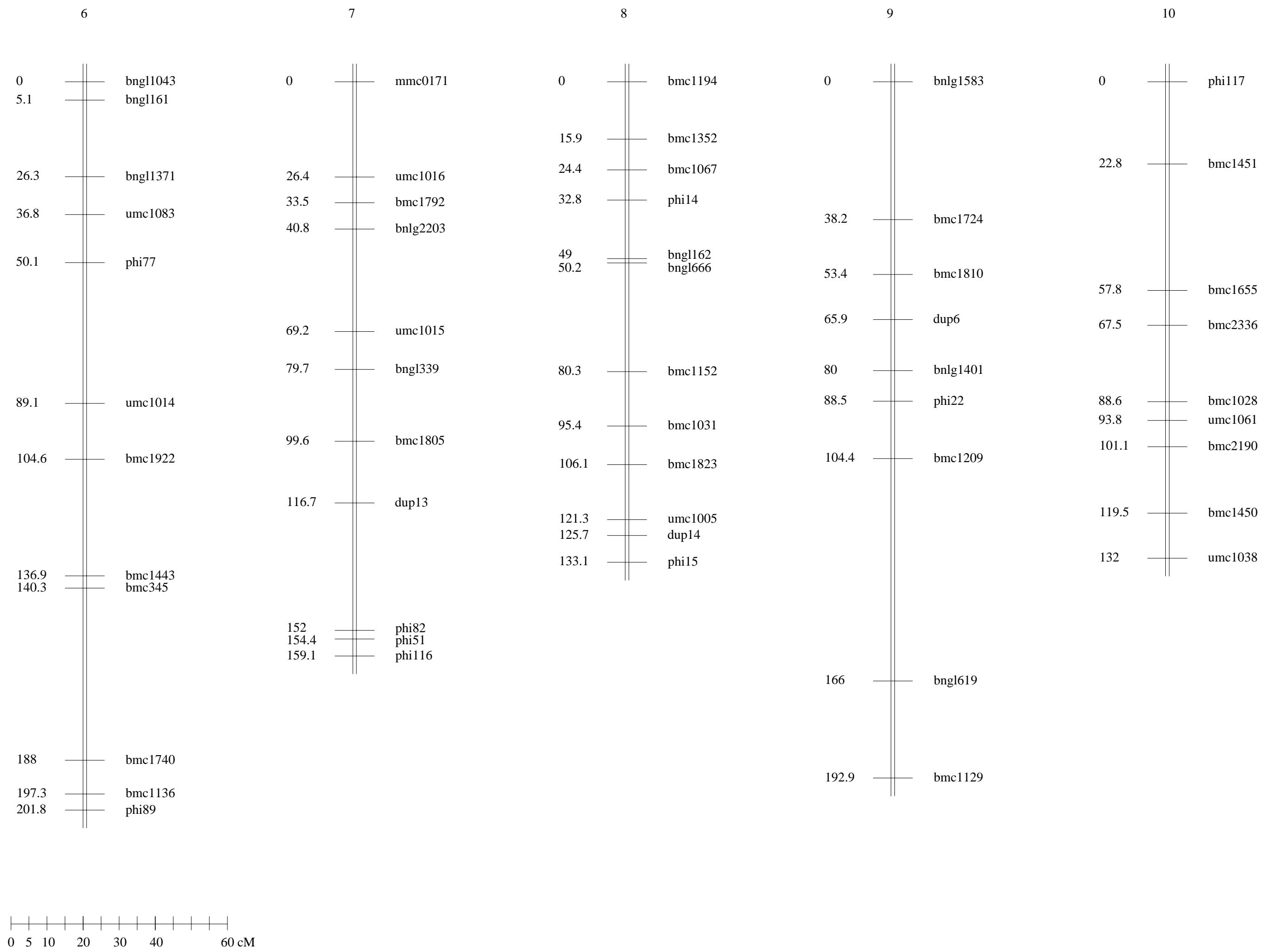

Figure 1. (Continued)

For all traits evaluated (FEM, MAS, ASI, YLD, PROLI, PH, and EH) in five environments, three acidic (V55, V65 and AYA65) and two normal-fertile soil (PAL and TUR), and based on the composite interval mapping analysis (CIM-model 4) with LOD=2.5, there were 66 QTLs identified across each environment. Thirteen QTLs were detected across acidic soils, 33 across normal-fertile soils, and 40 QTLs across all combined environments. In this study, no QTLs with major effects were identified. QTLs had low single and total R^2 values, for individual environments and across combined environments. The R^2 values, for each single QTL, ranged from 0.1% (COM. NS, chromosome 5 at 31 cM, bngl 143 SSR locus for PH, Table 27) to 15% (PAL, chromosome 6 at 95 cM, umc1014 SSR locus for PH, Table 27).

The total R^2 values ranged from 2% (COM. AS, and one marker for PROLI, Table 26) to 30% (COM. NS, and eight markers for PH, Table 27). Compared with data reported in the literature, these individual and total R^2 values are very small (small effects) in order to be used, individually, in a marker assisted selection program (Veldboom and Lee, 1996a; Veldboom and Lee, 1996b; Ribaut *et al*., 1996; Ribaut *et al*., 1997a).

Veldboom and Lee, (1996a,b) evaluated a population of 150 $F_{2:3}$ lines (derived from Mo17xH99) in two environments (stress and nonstress). RFLPs linkage map of 111 loci was used and QTL determinations were done for grain yield, yield components, plant height, and flowering. They concluded that the determination of QTLs based on the mean of environments were most informative than the QTL determination in a single environment. Based on those results we estimated the QTLs across five environments (three acid and two normal). The results for total phenotypic variance explained (R^2) across five environments (data not shown) were 7% for FEM, 10% for MAS, 1% for ASI, 3% for YLD, 4% for

Table 22. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the female flowering (FEM) expression at five environments (Env.): Combined for three acid soils (COM. AS) in Villavicencio with 55% (V55) and 65% (V65)Al saturation and Ayapel with 65% Al saturation. (AYA65); and combined for two normal soils (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia, 1998A.

Env.	Chromo-some	QTL (cM)	SSR locus	LR [1/] score	Add. [2/] days	Dom. [3/] days	R^2 [4/] (%)
V55	-	-	-	-	-	-	-
PAL	2	16	bmc 1017	12.02	0.50	-0.61	4
	3	192	bmc 1257	15.23	0.81	1.46	8
	6	6	bngl 161	14.21	0.71	0.13	4
	8	113	bmc 1823	12.84	0.49	-0.91	5
	9	172	bngl 619	12.21	0.51	0.69	4
Total							**24**
V65	7	69	umc 1015	12.84	-0.55	-0.33	3
		112	dup 13	13.37	0.56	0.43	3
Total							**3**
TUR	3	4	bmc 1523	12.73	0.32	-0.57	7
	9	98	bmc 1209	13.02	0.27	-0.81	4
Total							**11**
AYA65	1	138	dup 12	12.20	-0.93	-1.46	3
	3	162	mmc 0071	11.69	1.21	0.10	4
Total							**7**

Table 22. (Continued)

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] days**	**Dom. [3/] days**	**R^2 [4/] (%)**
COM. AS	7	69	umc 1015	18.95	-0.33	0.02	1
		116	dup 13	22.56	0.46	0.03	1
Total							**3**
COM. NS	1	183	mmc 0011	17.95	-0.72	-0.40	1
	3	162	mmc 0071	20.81	0.46	0.43	8
	4	210	mmc 0321	16.86	0.10	-0.23	2
	5	157	phi 101	16.70	0.02	-0.65	1
	8	113	bmc 1823	17.91	0.16	-0.67	5
	9	127	bmc 1209	15.78	0.80	-0.74	1
		173	bngl 619	20.32	0.27	0.35	4
Total							**22**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of “A” allele from parent 7 (tolerant) for allele “B” from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

PROLI, 4% for PH, and 15% for EH. Except for EH (15%) these values were lower than the total R^2 values for COM. NS (22, 20, 18, 5, 11, 30, and 10%). Additionally for yield, total R^2 across five environments was lower than both combined across environments (acidic and normal-fertile soils). These results clearly disagree with reports by Veldboom and Lee, (1996a,b). Because the QTL analysis across five environments (three acid soil, stress and two normal-fertile soil, nonstress environments) was not more informative, the results were not included.

QTLs were detected in all single environments for FEM, except V55 (Table 22). The single R^2 values were low and the largest total R^2 was in PAL (24%). There were a few common QTLs single environments and combined across environments. On chromosome 3 at 162 cM the SSR locus mmc 0071 was common at AYA65 and COM NS with R^2 of 4 and 8%, respectively. The QxE test rated mmc 071 as a stable QTL. Two QTLs were detected on chromosome 7 at 69 cM and at 112 cM (umc 1015 and dup 13) in V65 and COM. AS. These QTL were defined as unstable. For FEM in COM. NS 7 QTLs were identified, one on the chromosome 8 at 113 cM (bmc 1823) and other on the chromosome 9 at 173 cM (bngl 619). Both were common with PAL and rated as unstable. TUR and COM. NS had one common stable QTL. It was located around the marker bmc 1209 on chromosome 9. In TUR the QTL was identified at 98 cM and in COM. NS at 127 cM. The reason for two different positions possibly was because the biggest gap was found on this genomic region. QTLs identified for FEM, in this study, were at the chromosome 9 region (97cM to 123 CM), which was similar to previous studies (Ribaut *et al*., 1996).

Twelve QTLs were detected in all single environments for MAS (Table 23). The single R^2 values ranged from 0.2 to 10% and the largest total R^2 was in TUR (26%). There

Table 23. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the male flowering (MAS) expression at five environments (Env.): Combined for three acid soils (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soils (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia, 1998A.

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] days**	**Dom. [3/] days**	**R^2 [4/] (%)**
V55	3	66	dup 23	11.55	-0.53	-0.61	4
Total							**4**
PAL	2	19	bmc 1017	19.38	0.67	-0.65	7
	3	26	phi 29	11.80	0.53	-0.50	10
	8	112	bmc 1823	11.69	0.52	-0.73	6
Total							**21**
V65	4	3	bmc 1370	12.02	-0.60	1.17	3
	9	38	bmc 1724	16.94	-0.08	-0.76	8
Total							**11**
TUR	1	183	mmc 0011	18.11	-1.49	-0.72	0.5
	2	173	bnlg 1520	16.80	-0.55	-0.61	4
	3	162	mmc 0071	23.00	0.76	0.00	10
	4	117	umc 1031	15.49	-0.66	0.13	10
Total							**26**

Table 23. (Continued)

Env.	Chromo-some	QTL (cM)	SSR locus	LR [1/] score	Add. [2/] days	Dom. [3/] days	R^2 [4/] (%)
AYA65	5	120	bmc 1237	13.13	0.12	-2.20	5
	7	19	umc 1016	16.34	-1.24	-0.59	10
Total							**16**
COM. AS	7	17	umc 1016	20.38	-0.21	0.02	0.3
	9	38	bmc 1724	22.73	0.05	-0.50	2
Total							**2**
COM.NS	1	102	bmc 1273	19.27	-0.43	-1.07	2
		182	mmc 0011	21.05	-0.70	-0.39	2
	2	20	bngl 125	16.48	0.48	-0.52	7
	3	162	mmc 0071	24.84	0.53	0.16	2
	4	116	umc 1031	17.88	-0.52	0.33	5
	9	104	bmc 1209	16.53	0.95	-0.64	2
	10	24	bmc 1451	19.41	1.34	0.83	0.2
Total							**20**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of "A" allele from parent 7 (tolerant) for allele "B" from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

were a few common QTLs across single and combined environments. On chromosome 1 at 183 cM one QTL was identified near to mmc 0011 marker. This QTL was common in TUR and COM. NS. On chromosome 3 at 162 cM the SSR locus mmc 0071 was common at TUR and COM. NS with R^2 of 10 and 2%, respectively. The QxE test rated mmc 071 as an unstable QTL. Also this QTL was identified in FEM. Three QTLs were identified on chromosomes 2, 8, and 9 for MAS in PAL and COM. NS (normal-fertile environments). These QTLs were the bngl 125, bmc 1823, and bmc 1209, and they were located on chromosome 2 (at 20 cM), 8 (at 112 cM), and 9 (at 104 cM), respectively. Ribaut *et al.*, 1996 also identified the same QTLs for drought tolerance in intermediate (IS) and severe stress (SS). In COM. NS other QTLs for MAS were detected. On chromosomes 3 (at 162 cM), and 4 (at 116 cM) were identified the mmc 0071 and umc 1031, respectively. These QTLs were common in TUR. The mmc 0071 (R^2=10% in TUR) was detected also for FEM in AYA65 (R^2=4%). In COM. AS two QTLs common with V65 and AYA65 for MAS were identified. These QTLs (near at umc 1016 and bmc 1724 loci) showed very low R^2 values (0.3 and 2%, respectively). From QxE analysis the QTLs, nearest at bmc 1724, bngl 125, and umc 1031 were rated as stable QTLs.

Ten QTLs were detected in all single environments for ASI, except AYA65 (Table 24). The single R^2 values ranged from 1 to 7% and the largest total R^2 was in TUR (23%). One common QTL for ASI in PAL, TUR, and COM. NS was detected. It was located on chromosome 3 at 176, 167, and 171 cM (the nearest SSR locus was mmc 0071). The mmc 0071 locus presented R^2 values of 5, 7, and 5% at PAL, TUR, and COM. NS, respectively, was rated as unstable and it also was identified for FEM and MAS. In combined acid soil environments (COM. AS), one stable QTL on chromosome 6 at 94 cM (umc 014) and R^2 of

Table 24. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the anthesis silking interval (ASI) expression at five environments (Env.): Combined for three acid soils (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soils (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia, 1998A.

Env.	**Chromo-some.**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] days**	**Dom. [3/] days**	**R^2 [4/] (%)**
V55	5	151	phi 101	14.19	-0.21	-0.30	3
	9	98	bmc 1209	12.87	0.36	-0.20	4
Total							**7**
PAL	2	56	bmc 1537	12.66	-0.46	0.12	3
	3	176	mmc 0071	15.94	0.43	0.40	5
	4	220	mmc 0321	13.31	-0.38	0.30	6
Total							**14**
V65	7	58	umc 1015	15.03	-0.21	-0.39	4
Total							**4**
TUR	2	177	bnlg 1520	12.21	0.29	0.37	4
	3	167	mmc 0071	18.29	-0.39	0.39	7
	4	137	bmc 1621	12.71	0.30	0.42	7
	8	33	phi 14	12.29	0.42	-0.18	5

Table 24. (Continued)

Env.	**Chromo-some.**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] days**	**Dom. [3/] days**	**R^2 [4/] (%)**
TUR							
Total							**23**
AYA65	-	-	-	-	-	-	-
COM. AS	6	94	umc 1014	19.36	-0.22	-0.03	4
	9	84	bnlg 1401	19.70	0.04	0.01	2
Total							**6**
COM. NS	3	171	mmc 0071	28.89	0.01	0.37	5
	4	221	mmc 0321	15.39	-0.09	0.02	6
	6	51	phi 77	15.64	-0.59	0.30	1
	8	33	phi 14	16.38	0.01	-0.10	7
Total							**18**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of "A" allele from parent 7 (tolerant) for allele "B" from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

4% was identified. This QTL region was also reported for normal conditions (well watered, on chromosome 6 at 90 cM and R^2=11.3) by Ribaut *et al*., 1996. Other QTLs were detected in COM. NS that were common with PAL and TUR (mmc 0321 and phi 14, respectively). In V55 one QTL (chromosome 5 at 151 cM, phi 101) was detected that was also reported for drought tolerance in intermediate (IS) and severe stress (SS) by Ribaut *et al*. (1996).

Ten QTLs were detected in all single environments for YLD (Table 25). The single R^2 values ranged from 0.3 to 11% and the largest total R^2 was in PAL (19%). One QTL on chromosome 1 at 137 cM (dup 12) for YLD in AYA 65 was identified. This QTL with R^2 of 5% was also identified for PROLI (in AYA65 and COM. AS, Table 26), FEM (in AYA65) and EH (in TUR). Also, dup 12 was rated unstable for PROLI in COM. AS. In acid soils (COM. AS) for YLD only one QTL was detected: the SSR locus bmc1273 on chromosome 1 at 116 cM with R^2 of 7%, and it also was rated as unstable and common at V55 with R^2 of 8%. Three acid soil environments (V55, V65, and AYA65) and acid soil combined (COM. AS) presented regions of chromosome 1 related with QTLs for YLD at 103, 116, 137 and 181 cM with R^2 values of 8, 7, 5, 2%, respectively. From drought studies Ribaut *et al*. (1997a) also found on chromosome 1 for intermediate stress (IS), severe stress (SS),and combined environments regions at 82 and 156 cM with R^2 values of 5.6 and 7.7%. Chromosome 1 must be given greater consideration in future work.

Other QTLs were detected for YLD (Table 25). In normal-fertile combined environments (COM. NS) two QTLs were detected on chromosomes 4 and 8. The SSR loci umc 1031(R^2=2%) and bmc 1067 (R^2=3%) were located at 118 and 28 cM, respectively, and they were common in TUR. The bmc 1067 was rated as stable by QxE test. Also, umc 1031 position was similar to severe stress for drought tolerance (Ribaut *et al*., 1997a).

Table 25. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the yield (YLD) expression at five environments (Env.): Combined for three acid soils (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soils (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia, 1998A.

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] $t\ ha^{-1}$**	**Dom. [3/] $t\ ha^{-1}$**	**R^2 [4/] (%)**
V55	1	103	bmc 1273	13.27	0.05	-0.18	8
	2	96	bnlg 1887	12.25	-0.15	0.00	0.3
Total							**8**
PAL	2	2	bmc 1017	11.86	-0.39	0.10	5
	5	79	dup 10	13.30	-0.57	0.01	6
	6	19	bngl 1371	15.81	-0.57	0.01	11
Total							**19**
V65	1	181	mmc 0011	16.39	-0.45	-0.30	2
Total							**2**
TUR	4	118	umc 1031	12.59	0.27	0.10	5
	8	25	bmc 1067	14.95	0.33	0.20	5
Total							**10**
AYA65	1	137	dup 12	13.00	0.03	0.10	5
	5	100	bnlg 2323	12.39	-0.06	0.10	5
Total							**10**
COM. AS	1	116	bmc 1273	19.43	0.04	0.02	7

Table 25. (Continued)

Env.	Chromo-some	QTL (cM)	SSR locus	LR [1/] score	Add. [2/] t ha^{-1}	Dom. [3/] t ha^{-1}	R^2 [4/] (%)
COM. AS							
Total							**7**
COM. NS	4	118	umc 1031	18.86	0.23	0.17	2
	8	28	bmc 1067	18.75	0.29	0.27	3
Total							**5**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of "A" allele from parent 7 (tolerant) for allele "B" from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

QTLs for YLD and PROLI (yield component), were not common and consistent in single environments or combined across environments (acidic and normal-fertile). The percentage of phenotypic variance explained (R^2) was low and inconsistent. Considering only the acid environments the largest R^2 total was found at AYA65 for MAS with a value 16% and two QTLs on chromosomes 5 and 7 (at 120 and 19 cM, respectively).

The main target of this study was acid soils and yield, but 10% was the highest R^2 value at AYA 65, for two QTLs on chromosomes 1, and 5 (at 137 and 100 cM, respectively).

Table 26. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the ears per plant (PROLI) expression at five environments (Env.): Combined for three acid soils (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soils (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia 1998A.

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] No.**	**Dom. [3/] No.**	**R^2 [4/] (%)**
V55	-	-	-	-	-	-	-
PAL	2	0	bmc 1338	13.57	-0.07	0.01	2
		43	bmc 1537	17.74	0.10	0.01	5
	3	195	bmc1257	15.47	-0.12	-0.09	5
	6	201	phi 89	12.52	-0.06	-0.04	5
Total							**12**
V65	2	123	bmc 1258	12.70	0.08	0.00	2
Total							**2**
TUR	5	152	phi 101	12.68	-0.06	0.04	4
Total							**4**
AYA65	1	137	dup 12	16.36	0.05	0.07	6
Total							**6**
COM. AS	1	126	dup 12	20.47	0.01	0.01	2
Total							**2**

Table 26. (Continued)

Env.	Chromo-some	QTL (cM)	SSR locus	LR [1/] score	Add. [2/] No.	Dom. [3/] No.	R^2 [4/] (%)
COM.NS	2	0	bmc 1338	16.59	-0.03	0.02	2
		43	bmc 1537	20.03	0.07	0.01	5
Total							**11**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of "A" allele from parent 7 (tolerant) for allele "B" from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

For soil acidity combined across environments and for yield only one QTL was detected at chromosome 1 (at 116 cM) with a R^2 of 7%. Therefore, these results provided little evidence to support previous work with respect to aluminum tolerance related with a region on chromosome 8 (Reiter *et al*., 1991; Torres, *et al*., 1997). In this study, it was found in acid environments (single and combined) one QTL at 52 cM on the chromosome 8 in AYA65 for plant height (Table 27) with R^2 of 7% and another at 21 cM in COM. AS for ear height (Table 28), but only with R^2 value of 1%. Further work must be done to understand genetic relationship among PH, EH, and relative seminal root length (RSRL) in maize for acid soils.

Table 27. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the plant height (PH) expression at five environments (Env.): Combined for three acid soil (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soil (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia 1998A.

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] cm**	**Dom. [3/] cm**	**R^2 [4/] (%)**
V55	-	-	-	-	-	-	-
PAL	6	18	bngl 1371	12.70	4.81	-5.46	6
		95	umc 1014	16.80	6.57	1.35	15
	8	61	bngl 666	11.80	-1.33	7.38	2
Total							**20**
V65	1	180	mmc 0011	14.44	-6.06	-7.98	1
	5	61	bngl 105	12.06	-2.62	-2.40	2
		146	phi 87	12.02	2.83	2.20	4
Total							**8**
TUR	-	-	-	-	-	-	-
AYA65	4	89	phi 21	12.56	4.90	-6.23	3
	8	52	bngl 666	11.66	6.24	1.98	7
Total							**10**
COM.AS	5	149	phi 87	22.60	2.32	-0.11	0.5
		172	phi 85	19.56	-1.49	-0.65	1
Total							**4**

Table 27. (Continued)

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] cm**	**Dom. [3/] cm**	**R^2 [4/] (%)**
COM.NS	2	37	bngl 125	16.44	5.12	2.94	10
		88	bngl 1887	17.76	-2.14	-0.05	4
	4	38	bmc 1434	20.28	-13.40	-2.46	1
		212	mmc 0321	18.26	4.00	-3.48	6
	5	0	mmc 0151	15.78	-1.29	-3.70	3
		31	bngl 143	19.15	-8.23	0.38	0.1
	6	81	umc 1014	20.07	4.74	4.13	14
		107	bmc 1922	23.31	4.55	3.48	10
Total							**30**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of “A” allele from parent 7 (tolerant) for allele “B” from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

Table 28. QTLs characteristics (Chromosome, position in cM, and nearest SSR locus) related with the ear height (EH) expression at five environments (Env.): Combined for three acid soil (COM. AS) in Villavicencio with 55% (V55) and 65% (V65) Al saturation and Ayapel with 65% Al saturation (AYA65); and combined for two normal soil (COM. NS) in Palmira (PAL) and Turipaná (TUR), Colombia 1998A.

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] cm**	**Dom. [3/] cm**	**R^2 [4/] (%)**
V55	6	130	bmc 1443	11.95	1.22	-1.61	5
Total							**5**
PAL	2	48	bmc 1537	13.52	4.18	-2.16	7
	5	62	bngl 105	13.24	4.03	-2.51	4
Total							**10**
V65	1	178	mmc 0011	14.34	-3.41	-4.10	0.3
	6	90	umc 1014	14.82	2.15	-0.57	5
Total							**5**
TUR	1	138	dup 12	11.53	1.25	-3.80	4
	2	177	bngl 1520	11.82	2.96	0.91	3
Total							**6**
AYA65	6	26	bngl 1371	14.11	3.55	-2.16	6
Total							**6**
COM.AS	6	92	umc 1014	21.45	1.14	-0.44	1
		140	bmc 345	23.21	0.20	0.36	5
	8	21	bmc 1067	20.02	-0.33	0.72	1

Table 28. (Continued)

Env.	**Chromo-some**	**QTL (cM)**	**SSR locus**	**LR [1/] score**	**Add. [2/] cm**	**Dom. [3/] cm**	**R^2 [4/] (%)**
COM. AS							
Total							**6**
COM.NS	4	222	mmc 0321	17.27	1.52	-3.73	5
	5	60	bngl 105	22.47	-0.11	0.40	4
	9	158	bngl 619	16.63	1.24	-4.50	1
Total							**10**

[1/] LR score ≥ LR critical values at LOD=2.5 by CIM-model 4. LR Critical values of 11.51, 18.68, and 15.27 for single environment, COM. AS, and COM. NS respectively.

[2/] Additive effects (Add.) mean that the substitution effect of "A" allele from parent 7 (tolerant) for allele "B" from parent 8 (susceptible) tends to reduce (-) or to increase (+) the numerical value of the trait at this locus.

[3/] Dominance effects (Dom.) mean that the mean of heterozygous is less (-) or more (+) than the mean of two homozygous at this locus.

[4/] Phenotypic variance explained by each and all (total) detected QTL. The R^2 for each QTL will not sum up to the R^2 value from the multiple regression.

Results from this study and previous reports confirm the complexity of yield studies in maize. Additionally, QTLs analyses for yield in maize have identified QTLs in all ten chromosomes with wide variation in location, percentage of phenotypic variance explained, and effect sizes (Ribaut et al., 1997a). More specifically in this study, the complex acid soil showed that Al toxicity is important but it is not the only factor affecting maize yields. Lower yield tendency under acid soils, increased genotype by environment interactions, and decreased genetic variance affected the power of QTL detection.

In this study all traits were measured on plot (entry) basis. Additionally only morphological field traits were evaluated. In future work, data must be taken on an individual plant basis with greater accuracy (appropriate units) and reliable sample size. New variables from field or laboratory should be included such as RSRL, relative seminal root length value, obtained from the F_2 population grown in nutrient solution containing a toxic concentration of aluminum (Torres *et al*., 1997). Other variables that have already been tested by Urrea-Gomez *et al*., (1996) could be considered. These are fresh root weight, total length, or lateral root length in plants grown for 14 days in potted acid soil under greenhouse conditions with intermediate aluminum stress (45 to 65%). Current physiological studies, related with organic acids (citrate and itaconate) released for maize seedlings under aluminum toxic growth media, could provide more knowledge for tolerance to soil acidity in maize. Other topics to be considered include other mineral toxicities (Mn, Fe, etc), or mineral deficiencies (P, Ca, Mg, Mo, and Fe). Organic matter and acid soil interactions require more study and understanding.

Stuber *et al*. (1999) revised and compared the results of traditional plant breeding methods and marker-assisted selection in order to enhance breeding success. They concluded that use of both phenotypic and marker data will increase the rate of improvement. The relative efficiency of the data combination depends on heritability, genetic variance and scheme selection. Additionally, marker-assisted selection may become less efficient than phenotypic selection in the long term. Therefore, QTLs found, in this study, with small effects could be used in a carefully chosen selection index that, coupled with appropriate breeding scheme, could enhance traditional maize breeding success.

The information from this study is the basis for further work. We have SSR linkage map, seed of F_3 families, and some new knowledge to begin new projects.

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