Within-family genetic variation and epistasis in cassava (*Manihot esculenta* Crantz) adapted to the acid-soils environment.

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Abstract

Little is known about the inheritance of agronomic traits in cassava, nor on the relative importance of epistasis for most crops. A group of ten clones, adapted to the acid-soils environment was used as parents in a diallel study. Thirty genotypes were obtained from each F_1 cross and cloned. Each clone was represented by six plants, which were distributed in three replications at two contrasting locations. Genetic variability concentrated in the within-family component, which was statistically significant for all the variables analyzed (fresh root yield, fresh foliage yield, harvest index, root dry matter content, and plant type score) except for the reaction to super elongation disease (SED). Estimates of dominance variance were considerably larger than those of additive variance for fresh root and foliage yields. The reverse was observed for harvest index, dry matter content, plant type and SED scores. Epistasis played an important role only for fresh root and foliage productions. These results agree with those from similar studies targeting different environments. The common assumption of absence of significant epistatic effects common for many quantitative genetic designs is, therefore, challenged from the results for these two variables. Alternative breeding approaches are suggested according to the results obtained from this study.

Abbreviations:

CBB (cassava bacterial blight); CET (clonal Evaluation Trial); DMC (dry matter content); FRY (fresh root yield); FFY (fresh foliage yield); GCA (general combining ability); PTS (plant type score); SCA (specific combining ability); SED (score for the reaction to super elongation disease).

Introduction

Cassava (*Manihot esculenta* Crantz), is the fourth most important staple food after rice, wheat and maize and is a fundamental component in the diet of million of people (FAO/FIDA, 2000). Scott and collaborators (2000) estimated that for the 1995-97 period, annual production of cassava was about 165.3 million tons, with a value of approximately US$ 8.8 billion. Recent studies suggest that cassava is not only a reliable source of energy but also that it can be bred for enhanced nutritional quality in micronutrients and proteins (Chávez et al. 2004; Ceballos et al., 2004).

Few articles regarding the inheritance of quantitative traits in cassava have been published (Easwari et al. 1995; Easwari & Sheela 1998; Losada 1990). These studies suggested important non-additive gene action for root yield, number of roots, length of root, mean weight of root, dry matter content and the related starch content. Ironically, cassava shows in this regard a unique situation because a molecular map has been already developed (Fregene et al. 1997; Mba et al. 2001) along with valuable additional information in QTL detection (Cortes et al, 2002; Jorge et al. 2000; 2001; Okogbenin and Fregene, 2003) and yet it is complemented with limited knowledge on traditional genetics.

Cassava is an interesting crop because its vegetative propagation allows the estimation of within-family genetic variation and, indirectly, the relative importance of epistatic effects in relation to additive and dominance effects. Genetic studies analyzing the importance of epistatic effects are not very common, particularly in

annual crops. Adequate estimation of epistatic effects for complex traits, such as yield, is difficult and expensive. Holland (2001) published a comprehensive review on epistasis and plant breeding. Reports in the literature on the relevance of epistasis are infrequent and usually only for species that can be vegetatively propagated e.g. forest trees (Comstock et al. 1958; Foster and Shaw 1988; Isik et al. 2003; Rönnberg-Wästljung, & Gullberg 1999; Rönnberg-Wästljung et al. 1994; Stonecypher & McCullough 1986). Because of the complexities of these analyses and the costs involved, reports in the literature related to epistatic effects are frequently based on a limited number of genotypes sampled, giving variable if not contradictory results.

The cassava-breeding project at CIAT (International Center for Tropical Agriculture) is based on a mass phenotypic recurrent selection (Ceballos et al., 2004; Jennings and Iglesias, 2002; Hershey, 1984) and targets four major environments: sub-humid, acid-soils, mid-altitude valleys and highlands. Each environment is characterized by particular abiotic and biotic limiting factors. In the case of the acid-soil savannas, in addition to the edaphic problems of low pH, reduced Ca, P, and Mg availability and Al excess, disease pressure from super elongation disease induced by the fungus *Sphaceloma manihoticola* and cassava bacterial blight (CBB) induced by *Xanthomonas axonopodis* pv. *manihotis* are endemic in this region.

Because of the breeding scheme employed and the limited number of studies specifically designed to measure general (GCA) and specific (SCA) combining ability effects little information on the relative importance of additive and non-additive (dominance and epistasis) genetic effects in cassava. The objective of this study was to measure the within-family variation in a ten-parent diallel study conducted in two acid-soil environments to determine the relative importance of additive, dominance and epistatic genetic effects on the expression of several relevant traits of cassava.

Materials and methods

Griffing (1956) Method 4 diallel mating design was used to generate F_1 crosses among 10 parents. The inbreeding level of parental lines was considered negligible because no self-pollination has been involved in cassava breeding and crosses among related clones are generally avoided. Controlled pollinations were performed following the standard procedures described by Kawano (1980). As many parental clones as 31 were involved in the generation of new segregating populations targeting the acid-soil savannas (CIAT, 2001). These materials are generally selected based on their *per se* performance in this target environment. However, the 10 parents ultimately used in this study were those that allowed for a near balanced set of crosses. The botanical or true seeds were germinated and grown in a screen house until the seedlings were two-months old, when they were transplanted to the field at CIAT experimental station in Palmira, Valle del Cauca,

Colombia (Calle et al. 2004). F_1 plants were grown in the field for ten months. Among the many progenies (> 30) from a given F_1 cross, 30 were randomly chosen for this study based solely on their capacity to produce at least six good quality vegetative cuttings. Minor selection was thus unavoidable in determining the group of clones representing each F_1 cross in this study. The six stakes (clone) representing one genotype were distributed to the three replicates in each of two locations with different soil conditions, where the evaluations were conducted.

Both locations were at the Experimental Station in CORPOICA La Libertad near Villavicencio in Meta Department, Colombia (4° 06' N, 73° 29' W and at 400 meters above sea level). In spite of its proximity the two environments were very different. The *Loma* plot had severe edaphic constraints related to soil acidity (4.33 pH, 15.7 ppm P and 66.9% Al saturation). This site generally allows for high disease pressure from super elongation disease and CBB. Both diseases are endemic in this region and evaluation of the reaction of cassava to them is based on their natural incidence. The *Porcinos* site had much better soil conditions (4.73 pH, 24.7 ppm P and only 28.5% aluminum saturation). Before planting 0.5 t ha^{-1} of dolomite lime was applied to the soil. One month after planting the stakes 0.5 t ha^{-1} of 10-20-20 NPK fertilizer was applied following the standard recommendations for cassava grown in these environments.

A randomized complete block design with three replications was used in each location. The evaluation was similar to a split-plot design. Each replication contained 45 main plots, one for each of the 45 F_1 crosses of the diallel. Each F_1 cross was, therefore, randomly allocated within each replication. Main plots contained eight rows with seven plants per row. The first and last rows and the first and last plant within each row were filled with border plants (either of the parents for the respective F_1 cross). The rest of the plot (6 x 5= 30 subplots) was used to plant the experimental material. The 30 clones constituting each F_1 cross were planted together in the respective main plots of each replication. Row-to-row distances and separation of plants within row were 1 m for a final plant density of 10000 plants ha^{-1}. Trials were harvested in April 2002, ten months after planting (the usual age for harvesting cassava in this environment).

Plants were hand harvested individually. The roots, stems and foliage produced by each plant were weighted. Harvest index was measured as the ratio between root weight and total biomass. Root dry matter content was estimated using the specific gravity methodology (Kawano et al. 1987).

Reactions to diseases (super elongation disease and CBB), and plant type architecture were scored using a 1 to 5 scale where 1= resistant or excellent and 5= susceptible or very poor (CIAT 2002). Plant type score took into consideration several important characteristics such as plant vigor, erect architecture with few

branches and reduced branching angle, adequate capacity to produce vegetative cuttings, and amount of foliage present at harvest time.

Statistical model.

The analysis of variance was conducted following the expectations for each mean square described in Table 1. As is commonly the case, a few plants died or failed to develop normally to be harvested. To take into consideration this lack of uniformity, the harmonic mean was used as ***k*** (Venkovsky & Barriga 1992).

The total genetic variance has been partitioned into the between-family variation (σ^2_{F1}) and the within-family variation ($\sigma^2_{c/F1}$). The between-family variation, in turn, was partitioned into the well-known variances related to general (σ^2_{GCA}) and specific (σ^2_{SCA}) combining ability, which in turn allow the estimation of additive (σ^2_{A}) and dominance (σ^2_{D}) variances (Calle et al., 2005; Griffing 1956; Hallauer & Miranda 1988):

$$\sigma^2_{GCA} = (\text{Cov.HS}) = 1/4\sigma^2_{A} + 1/16\,\sigma^2_{AA} + 1/64\,\sigma^2_{AAA} + \text{..etc} \quad [1a]$$

$$\sigma^2_{SCA} = (\text{Cov.FS} - 2\,\text{Cov.HS}) = 1/4\,\sigma^2_{D} + 1/8\,\sigma^2_{AA} + 1/8\,\sigma^2_{AD} + 1/16\,\sigma^2_{DD}\text{...etc} \quad [1b]$$

where Cov.HS = covariance of half-sibs; Cov.FS = covariance of full-sibs; σ^2_{AA} = additive x additive epistatic variance; σ^2_{AD} = additive x dominance epistatic variance; σ^2_{DD} = dominance x dominance epistatic variance, and so forth.

Since parental clones are expected to have negligible levels of inbreeding $\sigma^2_A = 4*\sigma^2_{GCA}$ and $\sigma^2_D = 4*\sigma^2_{SCA}$ (Hallauer and Miranda, 1988). The estimates of additive and dominance variances are overestimated because they contain portions of epistatic variances (Equations 1a and 1b). Genetic parameters were estimated using the following mean squares from Table 1:

$$\sigma^2_{GCA} = [MS_{31} - MS_{32} - MS_{41} + MS_{42}] / rak\,(p-2) \quad [2a]$$

$$\sigma^2_{SCA} = [MS_{32} - MS_{42}] / rak \quad [2b]$$

Variance for these estimates were calculated as follows (Becker 1985; Vega 1987):

$$Var(\sigma^2_{GCA}) = \{2/[rak(p-2)]^2\}[(MS^2_{31}/df_{31}+2)+(MS^2_{32}/df_{32}+2)+(MS^2_{41}/df_{41}+2)+(MS^2_{42}/df_{42}+2)] \quad [3a]$$

$$Var(\sigma^2_{SCA}) = [2/(rak)^2]\ [(MS^2_{32} / df_{32}+2) + (MS^2_{42} / df_{42}+2)] \quad [3b]$$

In this evaluation, in addition to the usual between-family variation, the vegetative propagation of cassava allowed the analysis of the within-family variation. By cloning individual genotypes, they could be planted in two locations with three replications in each location. Therefore it was possible to partition the within-family variation into its genetic ($\sigma^2_{c/F1}$), genotype by environment ($\sigma^2_{c/F1*E}$) and the error (σ^2_e) components, as illustrated in Table 1.

The within-family analysis allows obtaining information on the relative importance of epistatic effects. The following equation has been developed when the assumption of negligible epistasis is true (Hallauer & Miranda 1988):

$$\sigma^2_{c/F1} - 3\ \text{Cov FS} + 4\ \text{Cov HS} \approx 0 \qquad [4]$$

The variance for this test is expected to be large (Hallauer & Miranda, 1988) because of the complexity of this linear function. The variance was estimated following the principles established in Lynch & Walsh (1998) and Isik et al. (2003), as follows:

$$\begin{aligned}\text{Var (Test)} &= \text{Var}[\sigma^2_{c/F1} - 3(\sigma^2_{SCA} + 2\sigma^2_{GCA}) + 4\sigma^2_{GCA}] \\ &= \text{Var}[\sigma^2_{c/F1} - 3\sigma^2_{SCA} - 6\sigma^2_{GCA} + 4\sigma^2_{GCA}] \\ &= \text{Var}[\sigma^2_{c/F1} - 3\sigma^2_{SCA} - 2\sigma^2_{GCA}] \\ &= \text{Var}(\sigma^2_{c/F1}) + \text{Var}(3\sigma^2_{SCA}) + \text{Var}(2\sigma^2_{GCA}) - 6\,\text{Cov}(\sigma^2_{c/F1}, \sigma^2_{SCA}) - \\ &\quad 4\,\text{Cov}(\sigma^2_{c/F1}, \sigma^2_{GCA}) + 12\,\text{Cov.}(\sigma^2_{SCA}, \sigma^2_{GCA}) \qquad [5]\end{aligned}$$

However, since $\text{Cov}(\sigma^2_{c/F1}, \sigma^2_{SCA}) = 0$ and $4\,\text{Cov}(\sigma^2_{c/F1}, \sigma^2_{GCA}) = 0$, the formula can be simplified:

$$\text{Var (Test)} = \text{Var}(\sigma^2_{c/F1}) + 9\,\text{Var}(\sigma^2_{SCA}) + 4\,\text{Var}(\sigma^2_{GCA}) + 12\,\text{Cov}(\sigma^2_{SCA}, \sigma^2_{GCA}) \qquad [6]$$

The last term in the equation can be estimated as:

$$\text{Cov}(\sigma^2_{SCA}, \sigma^2_{GCA}) = [(1/rak) * (1/rak(p-2)] * [\text{Cov}(MS_{32}, MS_{31}) - \text{Cov}(MS_{32}, MS_{32}) - \text{Cov}(MS_{32}, MS_{41}) + \text{Cov}(MS_{32}, MS_{42}) - \text{Cov}(MS_{42}, MS_{31}) + \text{Cov}(MS_{42}, MS_{32}) + \text{Cov}(MS_{42}, MS_{41}) - \text{Cov}(MS_{42}, MS_{42})]$$

in the above equation:

$$\text{Cov}(MS_{32}, MS_{31}) = \text{Cov}(MS_{32}, MS_{41}) = \text{Cov}(MS_{42}, MS_{31}) = \text{Cov}(MS_{42}, MS_{41}) = 0$$

$$\text{Cov}(MS_{32}, MS_{32}) = \text{Var}(MS_{32})$$

$$\text{Cov}(MS_{42}, MS_{42}) = \text{Var}(MS_{42})$$

Therefore,

$$\text{Cov}(\sigma^2_{SCA}, \sigma^2_{GCA}) =$$

$$= [(1/rak) * (1/rak(p-2)] * [- \text{Var}(MS_{32}) - \text{Var}(MS_{42}) + 2\,\text{Cov}(MS_{32}, MS_{42})] =$$

$$= -[2/(r^2a^2k^2(p-2)] * [(MS_{32})^2/(df+2) + MS_{42})^2/(df+2)]$$

Equation 6 can now be written as follows:

$$\text{Var}(\text{Test}) =$$

$$\text{Var}(\sigma^2_{c/F1}) + 9\,\text{Var}(\sigma^2_{SCA}) + 4\,\text{Var}(\sigma^2_{GCA}) -$$

$$-12\,[2/(r^2a^2k^2(p-2)]*[(MS_{32})^2/(df+2) + MS_{42})^2/(df+2)]$$

The standard error of the Epistasis Test for individual locations analyses is:

Var (Test One Location) =

$$\text{Var}(\sigma^2_{c/F1}) + 9\,\text{Var}(\sigma^2_{SCA}) + 4\,\text{Var}(\sigma^2_{GCA}) - 12\,[2/(r^2a^2k^2(p-2)]*[\,(MS_{32})^2/(df+2)]$$

The analysis of between-family variation was published elsewhere (Calle et al. 2004). In that article genetic effects, rather than genetic variances, were of interest and they were considered fixed effects. In the present study, however, the analysis of within-family variance and the relative importance of epistatic effect are of prime interest. All effects, therefore, were considered random and normally distributed. The 30 genotypes representing each F_1 cross are clearly a random sample of all the possible genotypes that could possibly be derived from the respective parents and contribute to most of the degrees of freedom in the analysis. The only criterion defining which genotype would be used was the capacity to produce six stakes in an environment different from the target environment where the evaluation was conducted. The progenitors in this study were among a group of 31 parental clones and the actual ten parents eventually involved were those that allowed for a balanced set of progenies. Therefore, the main criterion for the selection of the parental lines was their capacity to flower and produce adequate samples of botanical seed from many different crosses. That capacity varies from the environment where the F_1 plants were grown and that where the trials were conducted. The selection applied, therefore, is not expected to have had any bearing on plant architecture when plants were grown and evaluated in the acid-soils savanna environment.

The usual assumptions for this kind of analysis are: regular diploid behavior during meiosis; absence of cytoplasmic effects; linkage equilibrium, relatives are random members of a specified population and, because of the vegetative propagation of cassava, absence of C-effects (Libby & Jund 1962).

Results

In most F_1 crosses, 28 to 30 clones were actually evaluated in the field in each of the three replications at the two locations. Four crosses, however, were represented by a smaller number of clones (16, 18, 25 and 26, respectively) in the combined analysis across the two locations. In the analysis of results, therefore, k=27.96.

The two locations used in the evaluation showed significant differences for fresh root yield (FRY), fresh foliage yield (FFY) and plant type score (PTS) (Table 2). Genetic differences among crosses were found for dry matter content (DMC), PTS and reaction to super elongation disease (SED) but not for FRY, FFY and harvest index (Table 2). The error term for the differences among the F_1 cross averages is the respective genotype by environment interaction. These interactions were significant for all variables analyzed in the two environments. The magnitude of these interactions resulted in many main effects failing to reach statistical significance. A similar situation could be observed for the GCA and SCA

partitioning of the between-family variation (Calle et al., 2005), particularly for the case of GCA, which showed significant interactions with the environment for all variables as well (Table 2).

For the scores to the SED, GCA accounted for about 85% of the F_1 crosses sum of squares and SCA only the remaining 15%. Similar observations were made on different diallel studies (Cach et al. 2005; Jaramillo et al. 2004), in relation to prevalent pests in other environments (whiteflies, mites and thrips). PTS and DMC also showed a high dependency on GCA, which accounted for 82% of the sum of squares of F_1 crosses (Calle et al., 2005). Harvest index and FFY showed intermediate (not significant) dependency on GCA, which explained respectively 57 and 61% of the F_1 crosses sum of squares. GCA effects had the lowest impact in FRY, explaining only 42% of the F_1 sum of squares. Similar trends have been observed elsewhere (Cach et al. 2005; Jaramillo et al. 2005).

Since individual clone data has been included the degrees of freedom involved (Table 2) are considerably larger than those reported in the between family analysis (Calle et al. 2004). In every case, within-family genetic variation ($\sigma^2_{c/F1}$) was statistically significant (Table 2). The interaction between environment and the within-family genetic variation was also significant for all variables analyzed in the two locations, except for PTS. From the mean squares presented in Table 2 the estimates for σ^2_A, σ^2_D, and the test for epistasis (Table 3) were obtained as described above.

Variance components were considered important if the standard errors were less than half of the component estimates (Isik et al. 2003). Negative variances were considered to be zero, although their actual estimates were presented. Both, the reaction to SED and PTS, showed very distinctive results: genetic effects concentrated in the additive component, with considerably lower relevance of dominance effects and negative estimates for epistasis (Table 3).

Harvest index and DMC showed a similar pattern with σ^2_A larger than σ^2_D, and negligible epistasis, which in these cases, as opposed to PTS and SED, were positive (Table 3). The test for epistasis was statistically significant for FRY and FFY (Table 3). These two traits also showed a similar trend with considerably higher σ^2_A than σ^2_D but these estimates were not significant. Results from individual location analysis were similar to those in the combined analysis.

Discussion

The comparison of the relative magnitude of between- and within-family variation suggested that a large proportion of the genetic variability in cassava was detected as within-family variation for FRY and FFY (Table 3). These results reaffirm the observations made during the selection in evaluation trials where large number of crosses among elite parental lines, are represented by several clones. The within-family genetic variances for harvest index, DMC and PTS were larger than for between-family variation, but the difference was not as large as for FRY and FFY.

On the other hand, SED showed larger variation in the between- compared with the within-family component. Larger between-family variation was observed for reactions to thrips, white flies and mites in similar studies conducted in other environments and with a different set of parental lines (Cach et al. 2005; Pérez et al., 2005). The relatively simple inheritance for resistance to diseases or pests (with a strong dominance component) generate large variation between the averages of progenies involving one or two resistant parents compared with those from susceptible ones, with relatively little or no variation among the individual genotypes or clones within each family.

The cassava-breeding project at CIAT has recently started to generate data from the earlier phases of the selection process called Clonal Evaluation Trials (CET) that allow an estimation of the breeding value of parents used in generating these trials (Ceballos et al., 2004). In general, these estimations of breeding values based on the CET will be effective in traits were the genetic variation is concentrated in the between-family component or shows strong additive effects. Selection of outstanding parents for a given trait such as SED, will tend to generate uniform progenies also outstanding for that trait. This, in turn, could allow the implementation of the Backward GCA Selection described by Mullin and Park in 1992. For characteristics such as FRY, with strong non-additive effects and large within-family variation, the selection of outstanding parents would not be enough and individual clone analysis, within a given family, would be required.

In the present study, epistatic effects were important for FRY and FFY. This result agrees with those observed in similar studies conducted in distinctive environments, and involving mostly unrelated sets of parents (Cach et al. 2005; Perez et al. 2005) The magnitude of epistasis affecting FRY and FFY reveals the limitation of most quantitative genetic studies based on the assumption of negligible epistasis in such complex traits. The importance of epistasis revealed by these results also help to explain the difficulty in finding quantitative trait loci or QTLs accounting for the phenotypic variation observed in complex traits such as yield (Kao & Zeng 2002).

The mass phenotypic recurrent selection used for cassava genetic improvement exploits additive, dominance and epistatic genetic effects (Ceballos et al., 2004; Jennings and Iglesias, 2002; Hershey, 1984). However, the current breeding scheme cannot direct genetic improvement in such a way that the frequency of favorable genetic combinations (within or between loci) is efficiently maximized. To achieve this, special efforts to design parental clones (preferably with some degree of inbreeding) that produce better crosses and/or a reciprocal recurrent selection (Hallauer and Miranda, 1988), are required. The development of inbred clones specifically designed for their utilization as parents in breeding nurseries offers interesting advantages such as the possibility of a gradual and consistent assembly of favorable gene combinations, which in the current system occur just by chance. The important non-additive effects found in the present study justifies and supports this proposition.

Moreover, inbred parents offer additional advantages: a) Promote the discovery of useful recessive traits (e.g. waxy starch); b) Genetic load, which is expected to be high based on published results (Kawano et al. in 1978), could be reduced; c) Inbred parents would allow for the implementation of the back-cross scheme (Allard, 1960). This in turn will enhance the usefulness of valuable traits (e.g. genes for resistance to whiteflies, novel starch properties, etc.), which can then be transferred from one parental clone to another; d) Molecular markers and traditional genetic studies would be greatly facilitated; e) Germplasm exchange based on botanical seed is much easier than that of vegetative cuttings (Iglesias et al., 1994). This would promote south-to-south collaboration among the few cassava breeding projects of the world; and f) Cleaning planting stocks from viral or other pathogens could be achieved without the need of meristem culture.

The generation of doubled-haploids could reduce to two years the time required to attain desirable levels of inbreeding, which by successive self-pollinations would require about 10-12 years. CIAT is currently executing a project supported by the Rockefeller Foundation to develop the protocol for the production of doubled-haploids in cassava through anther culture.

The methodology and results of this study can be useful for other semi-perennial crops that can be vegetatively propagated. The diallel design employed, although cumbersome, it not very different from the traditional CET used in cassava

(Ceballos et al., 2004). The main differences were the restriction of a balanced set of crosses and the fact that each genotype (or clone) was planted in six, single-plant plots, rather than in a single six-plant plot. A balanced set of crosses allows for proper estimation of genetic parameters. The planting scheme allows a more precise estimation of the genetic value of each clone, because of the reduced impact of genotype x environment interaction. As a matter of fact, the data produced from this diallel study was used in the same way as that from CETs and the best clones were selected to continue in the following stages of selection (preliminary yield trials, advanced yield trials and regional trials).

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Table 1. Analysis of variance and expected mean squares for a 10-parents diallel design in which the 30 cassava genotypes representing each F_1 cross were clonally propagated[¶].

Source of variation	Degrees freedom	MS	Expected mean squares
Environment (E)	a-1	MS_1	
Rep/E	a(r-1)	MS_2	
F_1	[p(p-1)/2]-1	MS_3	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{F1*E} + rak\,\sigma^2_{F1}$
GCA	p-1	MS_{31}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rk(p-2)\,\sigma^2_{GCA*E} + rak\,\sigma^2_{SCA} + rak\,(p-2)\,\sigma^2_{GCA,}$
SCA	p(p-3)/2	MS_{32}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rak\,\sigma^2_{SCA}$
F1*E	(a-1)([p(p-1)/2]-1)	MS_4	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{F1*E}$
GCA*E	(a-1)(p-1)	MS_{41}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rk(p-2)\,\sigma^2_{GCA*E}$
SCA*E	(a-1)(p(p-3)/2)	MS_{42}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E}$
Error (a)	a([p(p-1)/2]-1)(r-1)	MS_5	$\sigma^2_e + k\,\sigma^2_\varepsilon$
Clones/F_1	(p(p-1)/2)(k-1)	MS_6	$\sigma^2_e + r\,\sigma^2_{c/F1*E} + ra\,\sigma^2_{c/F1}$
Clones/F_1*E	(p(p-1)/2)(k-1)(a-1)	MS_7	$\sigma^2_e + r\,\sigma^2_{c/F1*E}$
Error (b)	a(p(p-1)/2)(k-1)(r-1)	MS_8	σ^2_e

[¶]a= number of environments evaluated (2); r= number of replications within each environment (3); p= number of parents involved in the diallel crosses (10); k= number of cloned genotypes representing each F_1 cross (≈ 30). σ^2_e = Error associated with individual genotypes; $\sigma^2_{c/F1}$ and $\sigma^2_{c/F1*E}$ = genetic variation within F_1 crosses and its interaction with the environment, respectively; σ^2_ε = Error associated with the average of F_1 crosses; σ^2_{SCA} and σ^2_{SCA*E} = Specific combining ability variance and its interaction with the environment, respectively; σ^2_{GCA} and σ^2_{GCA*E} = General combining ability variance and its interaction with the environment, respectively; σ^2_{F1} and σ^2_{F1*E} = Variance between F_1 crosses and their interaction with the environment, respectively.

Table 2. Results of the analysis of variance in a ten-parent diallel cross experiment combining data from two different edaphic environments at CORPOICA – La Libertad (Villavicencio) in Meta Department, Colombia.

Source of variation	df	Fresh root yield (t ha^{-1})	Fresh foliage yield (t ha^{-1})	Harvest Index (0-1)	Dry matter content[¶] (%)	Plant type (1-5)	SED score (1-5)
Environm. (E)	1	475063.7**	731252.0**	5.62	3007.7	619.2**	n.a.
Rep/E	4	10594.4	30061.9	1.66	1389.5	16.9	43.85
F_1	44	1639.1	1685.8	0.38	420.3**	25.7**	21.82**
GCA	9	3413.0	4706.4	1.14	1683.0	103.6	90.53**
SCA	35	1182.9	909.1	0.18	95.6	5.7**	4.15
F_1*E	44	1362.3**	1463.5**	0.23**	167.0**	10.7**	n.a.
GCA*E	9	3532.6**	4171.3**	0.60**	578.9**	45.9**	n.a.
SCA*E	35	804.3	767.2**	0.14**	61.0*	1.7	n.a.
Error (a)	176	538.5	508.8	0.09	39.6	1.9	2.25
Clones/ F_1	1280	301.8**	444.115**	0.04**	25.0**	1.6**	0.52**
Clones/ F_1*E	1280	175.3**	212.8**	0.02**	5.7**	0.9	n.a.
Error (b)	4843	99.0	119.9	0.01	5.4	0.5	0.26

[¶] Because in some cases plants failed to produce enough roots to measure DMC, the degrees of freedom for this trait were Error (a) = 176; and Error (b) 4583.

Table 3. Variances and test for epistasis from the evaluation of a diallel set from ten parents combining data from two different edaphic environments at CORPOICA – La Libertad (Villavicencio) in Meta Department, Colombia. Within parenthesis the standard error for each parameter.

Genetic parameter	Fresh root yield	Fresh foliage yield	Harvest Index	Dry matter content	Plant type score	SED score
σ^2_G (Between F_1)	1.649 (2.954)	1.325 (3.094)	0.0010 (0.0006)	1.600 (0.664)	0.089 (0.039)	0.237 (0.055)
σ^2_G (Within F_1)	21.082 (2.297)	38.557 (3.242)	0.0030 (0.0003)	3.216 (0.169)	0.121 (0.012)	0.088 (0.066)
σ^2_A	-1.485 (6.321)	1.172 (8.035)	0.0015 (0.0016)	3.379 (2.399)	0.160 (0.144)	0.523 (0.234)
σ^2_D	9.028 (7.930)	3.384 (6.594)	0.0011 (0.0013)	0.873 (0.666)	0.096 (0.033)	0.092 (0.050)
Epistasis Test[¶]	15.054 (6.740)	35.433 (6.858)	0.0014 (0.0012)	0.872 (1.294)	-0.031 (0.077)	-0.242 (0.139)

[¶] Test for epistasis = $\sigma^2_{c/F1}$ – 3 Cov. FS + 4 Cov. HS