Epistasis in the expression of relevant traits in cassava (*Manihot esculenta* Crantz) for sub-humid conditions.

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Acknowledgemens

The technical assistance of Drs. B. Li and R. Macchiavelli for the statistical analysis of the within family variation is greatly appreciated. Any mistake in the analysis, however, is solely responsibility of the authors. This research was conducted with resources provided by the Ministry of Agriculture and Rural Development of Colombia.

Running Title: Epistasis in cassava

Abstract

There is limited knowledge on the inheritance of agronomic traits in cassava and the importance of epistasis for most crops. A nine-parent diallel study was conducted in sub-humid environments. Thirty clones were obtained from each F_1 cross. Each clone was represented by six plants, which were distributed in three replications at two locations. Therefore the same 30 genotypes of each F_1 cross were planted in the three replications at the two locations. Analysis of variance suggested significant genetic effects for all variables analyzed (reaction to thrips, fresh root and foliage yields, harvest index, dry matter content, and root dry matter yield). Significant epistatic effects were observed for all variables, except harvest index. Dominance variance was always significant except for dry matter content and dry matter yield. Additive variance was significant only for reaction to thrips. Results suggested that dominance plays an important role in complex traits such as root yield. The significance of epistasis can help to understand the difficulties of quantitative genetics models and QTLs in satisfactorily explaining phenotypic variation in traits with complex inheritance. Significant epistasis would justify the production of inbred parental lines to fix favorable allele combinations in the production of hybrid cassava cultivars.

Introduction

Cassava (*Manihot esculenta* Crantz), along with maize, sugarcane, and rice constitute the most important sources of energy in the diet of most tropical countries of the world. Cassava is the fourth most important basic food after rice, wheat and maize and is a fundamental component in the diet of million of people (FAO/FIDA, 2000). Scott et al. (2000) estimated that for the 1995-97 period, annual production of cassava was about 165.3 million tons, with a value of approximately 8.8 billion dollars ($US).

Little progress in understanding the inheritance of agronomic traits in cassava has been achieved. Few articles regarding the inheritance of quantitative traits have been published (Easwari et al. 1995; Easwari and Sheela 1998; Losada 1990). Cassava is perhaps unique in that a molecular map has been already developed (Cortes et al, 2002; Fregene et al. 1997; Jorge et al. 2000; 2001; Mba et al. 2001; Okogbenin and Fregene, 2003) but it is complemented with limited traditional genetics knowledge. Cassava is also an interesting crop because its vegetative propagation allows the estimation of within-family genetic variation and, indirectly, the relative importance of epistatic effects. Genetic studies analyzing the importance of epistatic effects are not very common, particularly in annual crops.

Accurate measurement of epistatic effects for complex traits, such as yield, is difficult and expensive. Reports in the literature on the relevance of epistasis are not as frequent as those estimating additive and dominance variances or effects and generally take advantage of the vegetative multiplication that some species offer (Comstock et al. 1958; Foster and Shaw 1988; Isik et al. 2003; Rönnberg-Wästljung, and Gullberg 1999;

Rönnberg-Wästljung et al. 1994; Stonecypher and McCullough 1986). In many cases these reports are on forest trees. 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.

Holland (2001) published a comprehensive review on epistasis and plant breeding. Several cases of significant epistasis have been reported in self- (Brim and Cockerham, 1961; Busch et al. 1974; Gravois, 1994; Hanson and Weber, 1961; Pixley and Frey, 1991; Orf et al., 1999) and cross-pollinated (Ceballos et al.,1998; Eta-Ndu and Openshaw, 1999; Lamkey et al., 1995; Melchinger et al., 1986; Wolf and Hallauer, 1997) crops. According to Holland (2001) finding significant epistasis seems to be easier in self- than in cross-pollinated species and in designs based in the contrasts of means rather than the analysis of variances.

The objective of this study was to analyze the within-family variation in a diallel study conducted in two sub-humid environments and to assess the relative importance of additive, dominance, and epistatic genetic effects on the expression of several relevant traits of cassava.

Materials and methods

A diallel mating design was used to generate F_1 crosses among 9 parents. Inbreeding level of parental lines was considered zero because no self-pollination has been involved in cassava breeding and crosses among related clones are generally avoided.

Kawano et al. (1978) provided evidence that cassava is a highly heterozygous species. Controlled pollinations were performed following the standard procedures described by Kawano (1980). Many parental clones were initially involved but the parents ultimately used (as well as the number of parents involved) were those that allowed for as balanced a set of crosses as possible. Botanical seed 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. F_1 plants were grown in the field for ten months. Among the many genotypes (> 30) from a given F_1 cross, 30 were randomly chosen for this study based solely on their capacity to produce at least six vegetative cuttings. Each of these stakes was planted in one of three replications at one of two locations.

Trials were planted during July 2001 in two sub-humid locations in Colombia (Cach et al. 2005). A randomized complete block design was used. The evaluation was similar to a split-plot design. Each replication contained 36 main plots, one for each of the 36 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. The rest of the plot (6x5= 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. The experimental design, therefore, offered two types of error: (a) associated with the main plots or F_1 averages, and (b) the error associated with the sub-

plots or within-F_1 variation. Row-to-row distances and separation of plants within row were 1 m for a final plant density of 10000 plants ha^{-1}.

The six vegetative cuttings obtained from each plant in the nursery at Palmira were distributed in the three replications at the two locations for the evaluation trials. Therefore for each F_1 cross, the same group of 30 genotypes was used in each experimental plot. Trials were harvested in May 2002, ten months after planting (the usual age for harvesting cassava in this environment). One month after planting 330 kg ha^{-1} of a 15-15-15 NPK fertilizer was applied to the soil, following the standard recommendations for cassava grown in this region of Colombia.

Plants were hand harvested individually. The roots produced by each plant were weighted as well as the above ground biomass (stem and foliage). 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). Approximately three to five kilograms of roots were weighed in a hanging scale (WA) and then, the same sample, was weighed with the roots submerged in water (WW). Dry matter content of the roots produced from each plant was estimated individually utilizing the following formula:

$$\text{Dry matter content (\%)} = \{[WA / (WA-WW)] * 158.3 \} - 142$$

where WA= weight in the air and WW= weight in water.

Reaction to thrips (*Frankliniella williamsi*), plant type architecture and general root appearance 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, amount of foliage present at harvest time and absence of foliar diseases (which in this particular environment are not frequent).

Statistical model.

The analysis of variance was conducted following the expectations for each mean square described in Table 1. The analysis takes advantage of the full- (FS) and half-sib (HS) families that the diallel mating design creates. As is commonly the case, a few plants died or failed to develop normally to be harvested. Therefore in a few F_1 crosses fewer that 30 clones were actually evaluated in the field in each of the three replications at the two locations. To take into consideration this lack of uniformity, the harmonic (not the arithmetic) mean was used as ***k*** in the expected mean squares formulas (Vencovsky and Barriga 1992; see bottom of Table 2). The total genetic variance was partitioned into 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 σ^2_A and σ^2_D (Griffing 1956; Hallauer and Miranda 1988):

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

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

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 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 environmental (σ^2_e) components, as illustrated in Table 1.

The within-family analysis allows estimation of the relative importance of epistatic effects. In the absence of epistasis the following equation holds true (Hallauer and Miranda 1988):

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

Therefore, a test statistics for the significance of epistatic variance can be constructed by using estimates of the parameters on the left side of the equation. The variance for this test statistic is expected to be large (Hallauer and Miranda, 1988) because of the complexity of this linear function. The variance was estimated following the principles established in Lynch and Walsh (1998) and Isk et al. (2003), as follows:

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

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

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

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 estimates of σ^2_{GCA} and σ^2_{SCA} additive and dominance variances but these estimates are biased upward because they contain portions of epistatic variances (Equations 1a and 1b).

The analysis of between-family variation was published elsewhere (Cach et al. 2005). 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 possible genotypes that could possibly be derived from the respective parents. 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 parents involved in this study were among a group of 25-30 clones characterized by their adaptation to sub-humid conditions: long periods without rain, tolerance or resistance to insect and arthropod pests typical for these environments (particularly thrips and different species of mites), and a frequent susceptibility to foliar diseases (because they are not common in this kind of environment). Eight of the parents evaluated come from CIAT's cassava-breeding project in Colombia and the remaining clone was a cultivar released many years ago in Thailand. These parents are considered to be part of a reference population of clones adapted to the sub-humid, lowland, tropical environment.

The actual nine parents eventually included were those that allowed for a balanced set of progenies for the study. 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. It is difficult to assess the impact (if any) of this selection because crossings are made in the mid-altitude valleys environment where CIAT headquarters are located, but the evaluation was conducted in a completely different environment. This is important because the flowering habit, which profoundly affects plant architecture vary drastically from one environment to the other. A non-branching, erect type in the sub-humid environment may be bushy and flower profusely at Palmira. Because of this situation it can be assumed that the effect of selection of parents at Palmira had a neutral impact on the general performance of the progenies selected and evaluated for this study.

The analysis of variance for the between-family variation follows the method 4 proposed by Griffing (1956). The usual assumptions for Method 4 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, negligible C-effects (Libby and Jund, 1962). In the case of cassava, C-effects would result from differences in the physiological/sanitary status between F_1 mother plants and/or among the six stakes used to clone each genotype and these differences would be confounded with the environmental and/or genotype x environment interactions components of variation. Since the F_1 plants from which the six stakes were taken had been grown in Palmira under excellent management

practices, differences (if any) in the physiological/sanitary status of these vegetative cuttings are reasonably expected to be small and negligible.

Results

The coefficients of variability indicated that the experimental error involved in this study was relatively low. Results, therefore are reliable and the precision of the analysis, acceptable (Cach et al. 2005). The two locations used in the evaluation showed statistical differences only for foliage yield and harvest index (Table 2). On the other hand statistical differences among crosses were found for all the variables analyzed. With the exception of the plant type score, the crosses by environment interactions were also significant. GCA mean squares were significant for all variables except harvest index (Table 2). SCA mean squares were also significant for all variables except harvest index and dry matter yield.

Since individual clone data has been included, the degrees of freedom involved are considerably larger (Table 2) than those reported in the between family analysis (Cach et al. 2005). In every case, within-family genetic variation ($\sigma^2_{c/F1}$) was statistically significant. The interaction between environment and the within-family genetic variation also proved to be statistically significant. From the mean squares presented in Table 2 the estimates for σ^2_A, σ^2_D, and the test for epistasis 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). The estimate for σ^2_D was larger than that for σ^2_A for fresh root and foliage yields, harvest index and dry matter yield and smaller for reaction to thrips and dry matter content (Table 3). Epistasis was highly significant for all variables (test values > two times the value of their respective standard errors) except harvest index (Table 3).

Discussion

Based on the magnitude of the estimates for between- and within-family genetic variances, a large proportion of the genetic variability (79-93%) remained as within family variation (Table 3). These results agree with observations during the selection in evaluation trials where large numbers of crosses among elite parental lines are represented by several clones. As expected, the lowest within-family variation (79% of total genetic variance) was measured for a relatively simply inherited trait such as the reaction to thrips (Bellotti 2002), which showed the only statistically significant additive variance. The tolerance/resistance in outstanding parents transmitted to the progeny tended to accentuate differences among families and reduce the variability among sister clones. A similar situation was observed in a similar study for the mid-altitude valleys environment (Pérez et al., 2005). However, it is clear that a considerable within-family variation still remained even for the reaction to thrips. On the other hand, complex traits such as root and foliage yields showed a larger partitioning of the total genetic variance

(> 90%) into the within-family variation, suggesting that there were, comparatively, smaller differences in the breeding values of the progenitors.

The within-family variation suggested not only important genetic effects, but also significant genotype-by-environment variation for all variables analyzed. This interaction implies that reliable selection can only be made when enough planting material for replicated trials at more than one location, has been produced. In practice, this means the third or fourth stage in the selection process (Ceballos et al., 2004). One alternative for overcoming this problem would be to modify the clonal evaluation trials (first stage in the selection process), which currently is conducted as an unreplicated trial at a single location, with seven plants per genotype (Ceballos et al., 2004). The total number of plants per genotype can be raised to eight so that two trials, at two different locations, and with four plants per genotype at each location can be planted. Although the costs related to this change are large, and the logistic complications considerable, the data provided by this experiment (and other similar studies) suggest that they may be justifiable.

Dominance effects were very important for thrips, harvest index and root and foliage yields, with variance estimates significantly different from zero (estimates two times or more the size of the respective standard error). Only the score for thrips and dry matter content showed larger estimates for the additive compared with the dominance variance (Table 3). This highlights the importance of heterosis in cassava breeding for many

relevant traits, which in turn justifies the implementation of a reciprocal recurrent selection scheme for cassava genetic improvement.

Epistatic effects were significant for all variables, except harvest index, based on the test for epistasis (Table 3). It was surprising to see the size and generalized significance of epistatic effects. In many cases reported in the literature, epistatic effects may have been large but failed to reach statistical significance, in part, because of the size of the standard errors typical for complex linear functions (Hallauer and Miranda 1988; Hinze and Lamkey, 2003; Holland, 2001). In this study, however, this was not the case. To a large extent this may be the result of the large size of this experiment, which resulted in large degrees of freedom for the overall analysis, including the number of clones within family and the number of replications and environments employed. However, the large and frequent epistasis found in this study may also be the result of the evolutionary history of this species that can multiply both sexually or clonally. It is feasible that cassava has evolved to take advantage of favorable gene combinations resulting from dominance and epistatic relationships by fixing them through the vegetative mode of reproduction. The results of this study reveal the limitation of most quantitative genetic studies based on the assumption of negligible epistasis. These results would also help to explain the difficulties in finding QTLs that satisfactorily explain the phenotypic variation observed in complex traits such as yield (Kao and Zeng 2002).

The phenotypic clonal selection used for cassava breeding takes advantage of the vegetative reproduction of the crop. In selecting outstanding clones all genetic effects

(additive, dominance and epistatic) are exploited (Ceballos et al., 2004; Mullin and Park, 1992). However, the current recurrent selection system lacks the capacity to direct genetic improvement in such a way that the frequency of favorable (within or between loci) genetic combinations is maximized. To achieve this, special efforts to design parental clones that produce better crosses are required.

CIAT has recently introduced modifications that allow for the estimation of GCA effects in early stages of the selection process (Ceballos et al., 2004). This, in turn, allows the implementation of the Backward GCA Selection described by Mullin and Park in 1992. Results from this study suggest that this approach would be ideal for traits such as the reaction to thrips given the importance of GCA effects and the comparatively low relevance of dominance and epistatic effects. For complex traits such as fresh-root yield, however, the prevalence of non-additive effects suggested by this study, would require a different approach. The development of clones specifically designed for their utilization as parents in breeding nurseries would be one alternative that offers interesting advantages. Introduction of inbreeding in these parental clones would facilitate the gradual and consistent assembly of favorable gene combinations, which in the current system occur just by chance. Inbreeding would also facilitate the reduction of the genetic load of this crop, which is expected to be relatively large at this point in time.

One major constraint for the introduction of inbreeding in cassava is the time required for it. The production of doubled haploids through anther or microspore culture is an

interesting approach that would reduce the time required to obtain homozygous genotypes. This, in turn, will maximize the exploitation of dominance and epistatic genetic variation, which have been found to be significant in this study. CIAT is currently executing a project financed by the Rockefeller Foundation to develop the protocol for the production of doubled-haploids in cassava.

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Table 1. Analysis of variance and expected mean squares for a 9-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	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{F1*E} + rka\,\sigma^2_{F1}$
GCA	p-1	MS_{31}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rk(p-2)\,\sigma^2_{GCA*E} + rka + \sigma^2_{SCA} + + rka(p-2)\,\sigma^2_{GCA}$
SCA	p(p-3)/2	MS_{32}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rka\,\sigma^2_{SCA}$
F_1*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 (9); k= number of cloned genotypes representing each F_1 cross (30).

Table 2. Mean squares from the analysis of variance in a diallel set from nine parents combining data from two locations (Pitalito and Sto. Tomás) in Atlántico Department, Colombia.

Source of variation	df	Thrips score 1-5	Fresh root yield t ha^{-1}	Fresh foliage yield t ha^{-1}	Harvest Index 0-1	Dry matter content %	Dry matter yield t ha^{-1}
Environm. (E)	1	32.6^{NS}	8901.1^{NS}	191775.7**	7.331*	3370.0^{NS}	2508.4^{NS}
Rep/E	4	36.4	2010.0	6273.0	0.595	608.3	361.2
F_1	35	39.3**	3206.1**	2896.3**	0.262**	192.8**	222.6*
GCA	8	136.0**	8516.3^{NS}	7535.6^{NS}	0.587^{NS}	612.2*	537.2^{NS}
SCA	27	10.6**	1632.7**	1521.6**	0.166**	68.6^{NS}	129.3^{NS}
F_1*E	35	2.0^{NS}	1040.1**	989.8**	0.093**	65.6**	108.2**
GCA*E	8	5.1**	2371.1**	2966.1**	0.228**	162.0**	257.9**
SCA*E	27	1.1^{NS}	645.8^{NS}	404.3**	0.053*	37.1**	63.9^{NS}
Error (a)	140	1.2	442.2	477.8	0.031	18.8	37.6
Clones/F_1	1014	4.2**	1005.7**	985.0**	0.029**	41.5**	80.3**
Clones/F_1*E	1014	0.4**	242.4**	193.8**	0.007**	8.2**	20.5**
Error (b)	3789	0.3	175.5	126.9	0.006	5.8	14.7
Overall error	3929	0.3	185.0	139.4	0.007	6.3	15.6
k[¶]		27.6	27.6	27.6	27.6	27.5	27.5

[¶] Harmonic mean for numbere of genotypes within F_1 families.

Table 3. Variances and test for epistasis from the evaluation of a diallel set combining data from two locations (Pitalito and Sto. Tomás) in Atlántico Department, Colombia. Within parenthesis the standard error for each estimate.

Genetic parameter	Thrips (1-5)	Fresh root yield	Fresh foliage yield	Harvest Index	Dry matter content	Dry matter yield
σ^2_G (Between F_1)	0.225	13.09	11.53	0.0010	0.772	0.694
σ^2_G (Within F_1)	0.641	127.21	131.86	0.0037	5.556	9.977
σ^2_G (Total)	0.867	140.30	143.39	0.0048	6.328	10.671
σ^2_A	0.419 (0.211)	17.82 (13.75)	11.93 (12.59)	0.0009 (0.0010)	1.452 (0.985)	0.741 (0.933)
σ^2_D	0.231 (0.068)	23.87 (11.15)	27.02 (10.00)	0.0027 (0.0011)	0.765 (0.497)	1.589 (0.919)
Epistasis Test[¶]	0.259 (0.119)	100.40 (12.74)	105.64 (11.84)	0.0013 (0.0009)	4.257 (0.673)	8.414 (0.990)

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

1