OUTPUT 9
Activities related with the maintenance of the germplasm bank of cassava and other *Manihot* species. Basic genetic studies.

CIAT has been trusted with the maintenance of the cassava world germplasm bank, which includes more than 6000 accessions of *Manihot esculenta* and other *Manihot* species. In the following pages a summary of activities related to the germplasm bank and other basic genetic studies will be described. It is important to emphasize that all these activities are also reported as part of the joint work between IP3 and SB2 projects. The inclusion of this Output in the IP3 Annual Report is just to provide a whole picture of research and results around cassava at CIAT.

Activity 9.1. Maintenance of Manihot germplasm bank in the field.

Rationale

The Genetic Resources Unit is officially in charge of the maintenance of the cassava germplasm bank, both *in vitro* and in the field. However, for practical reasons, the field operations are coordinated by IP3 project. Since year 2000 an extensive activity to clean up from frogskin disease, the germplasm bank has been carried out. Plots from the germplasm bank maintained in the field, because of its very nature, could not be eliminated even if frogskin disease appeared in some of the plants. Eventually the incidence of the disease increased to unacceptable levels.

In order to reduce the costs of maintenance of the germplasm collection and because of the problems associated with frog skin disease it was decided that the collection will be moved from the field and be maintained using the "bonsai system" under greenhouse conditions. This is an activity described in more detail in the respective report from the Germplasm Collections and will not be further discussed herein.

Because the Germplasm Collection is no longer in the field during this period, a set of clones has been regenerated in order to produce enough roots for the evaluation of different traits, particularly novel starch quality traits. IP3 project is trying to produce plants from as many as 2000 clones from the germplasm collection. These plants are produced from stocks that have been certified to be frog skin free. In addition the core collection (about 600 clones) has been shipped to Thailand so there is a duplicate of this important group of clones. The core collection will be phenotypically characterized in Thailand and also in Colombia. This evaluation will allow a measurement of the relative stability or sensitivity to genotype by environment interaction of the morphological descriptors used in the Genetic Resources Unit for cassava.

Specific Objectives:

a) *To grow plantlets from the in vitro core collection.*
b) *To grow plantlet from in vitro plants of the germplasm bank for starch quality evaluations.*

Results

We have begun a systematic characterization of the starch properties in the roots of the accessions from the germplasm bank. Every year up to 2000 accessions are evaluated. So far approximately 4000 clones have been characterized in the last few years and a group of about 2000 clones will be evaluated next year. To do this evaluation plants from these clones have been gradually recovered from the in vitro collection. The results of this evaluation will be published as soon as the data set is completed and an agreement has been reached with the company financing this research. To produce the required plants the following steps need to be taken.

Regeneration of each accession from the in vitro collection.

From each accession, a plant from the *in vitro* collection was regenerated and indexed to certify it is free of diseases. Plants passing this first test are then hardened in conditions that do not allow for the presence of white flies, and therefore, minimizes the possibility of acquiring the frogskin disease agent again.

Because of the higher incidence of frogskin disease at CIAT plants that are certified to be disease free, or those developed from botanical were planted outside CIAT in isolated plots (CEUNP). Only virus-free plants were planted in those isolated plots. In the meantime, plantings at CIAT were reduced as a higher proportion of the cassava germplasm is being certified to be disease-free. In short the outside plantings were certified to be *"clean",* whereas the plantings at CIAT were not. This situation was maintained until the middle of 2001, when materials not certified to be disease free moved out of CIAT, and those that are *clean*, came back to the station.

In addition of maintaining an ideal reservoir for the agent of the frogskin disease in the germplasm bank, there is a second factor that facilitated the spread of the disease. In effect, the white flies problem has increased considerably during the last few years. A major factor for this increment has been the continuous planting of cassava year round. The insects, therefore, had an ideal condition for maintaining high population densities. Between June 1 and June 30, 2003, there was no cassava plant in the field at CIAT's station in Palmira. It is expected that this measure will reduce population densities for the insect, and in turn, will reduce to a minimum the already inefficient transmission of the frogskin disease agent to healthy plants.

A common procedure to harvest cassava is to first take the stakes (vegetative *"seed"*) out of the field, and then harvest the roots. In fact this practice prevents the elimination of stakes from diseased plants, because when the roots are evaluated for symptoms, the stakes from each plant has already been mixed with other stakes from different plants. Starting in this year, the harvest protocol has been changed slightly. The whole plant is first taken out of the ground, so before taking the stakes the roots can be inspected to make sure they are asymptomatic. Stakes are taken only from plants that do not show the symptoms. This practice will reduce to a very minimum the "seed" transmission of the disease to only two possible cases: **a)** when the worker fails to recognize the symptoms; or **b)** when the plant has been infected late in the season and, therefore, it does not show the symptoms but the disease will be transmitted through its stakes.

All the activities were carried out as expected. A large proportion of accessions from the germplasm bank was evaluated for frogskin disease and, if clean, planted in isolated conditions. Sequential plantings were performed as the plants were certified to be disease-free. Therefore, harvest of these plants was also done sequentially. The levels of frogskin were very low, as expected. However, given the results from the previous year, when higher than acceptable levels of frogskin disease were observed, it has been decided not to plant the entire germplasm bank in the field, until the vector(s) and pathogen(s) are clearly determined. At the end of October a total of 228 genotypes from the core collection have been hardened in screen house conditions (Figure 9.1), with a total of 975 plants (average of 4.3 plants/genotype). Vitroplants came in batches and that is why the four plantlets at the right bottom of Figure 9.1 show different developments.

Figure 9.1. Illustration of the process to harden vitro plants followed for the recovery of genotypes from the core collection for their evaluation in the field.

Activity 9.2. Evaluation of* M. esculenta *and related species from the germplasm collection for useful traits, particularly for higher protein content in the roots.

Rationale

Many of the activities related to the evaluation of the introgression of useful genetic variability from wild relatives of cassava is described in Otuput 12 and also in Output 1. In this activity, however, a particular action will be described because of its relevance. In a previous Annual Report (2002) it was reported that a few clones more commonly from Central America had been found to have high levels of crude protein in the roots. These clones were properly identified and were recovered from the germplasm collection to be evaluated again to confirm their protein content in the roots. One important feature that will be evaluated is the stability of that trait through multi-location evaluations and also the effect of the age of the plant.

Specific Objectives:

a) *To grow plants recovered from the in vitro collection and measure protein content in their roots.*

Results.

Plants from 21 clones were hardened and grown in the field. At eight months of age one root was taken from the plants in the field and flour obtained from them. N content was measured at CIAT's analytical laboratory and multiplied by the standard 6.25 factor to obtain % of crude protein content in the roots. Figure 9.2 shows the results from these measurements (at eight months of age) as well as the original data from a 1999 evaluation on 10-month old plants.

The results presented in Figure 9.2 are preliminary. The fact that the quantifications were made at two different laboratories should not have an effect based on the results presented in Output 1. The major difference between the two evaluations is the age of the plants. These plants will be kept in the field for few more months and toots taken at 10 months of age for yet another quantification of protein content. In spite of the obvious disagreement between the two analyses depicted in Figure 9.2, it is obvious that at least five clones showed again protein levels above 5%, which is about twice as much as the content traditionally considered for cassava. These plants are in the field to be crossed and self-pollinated, and constitute a very valuable and promising germplasm.

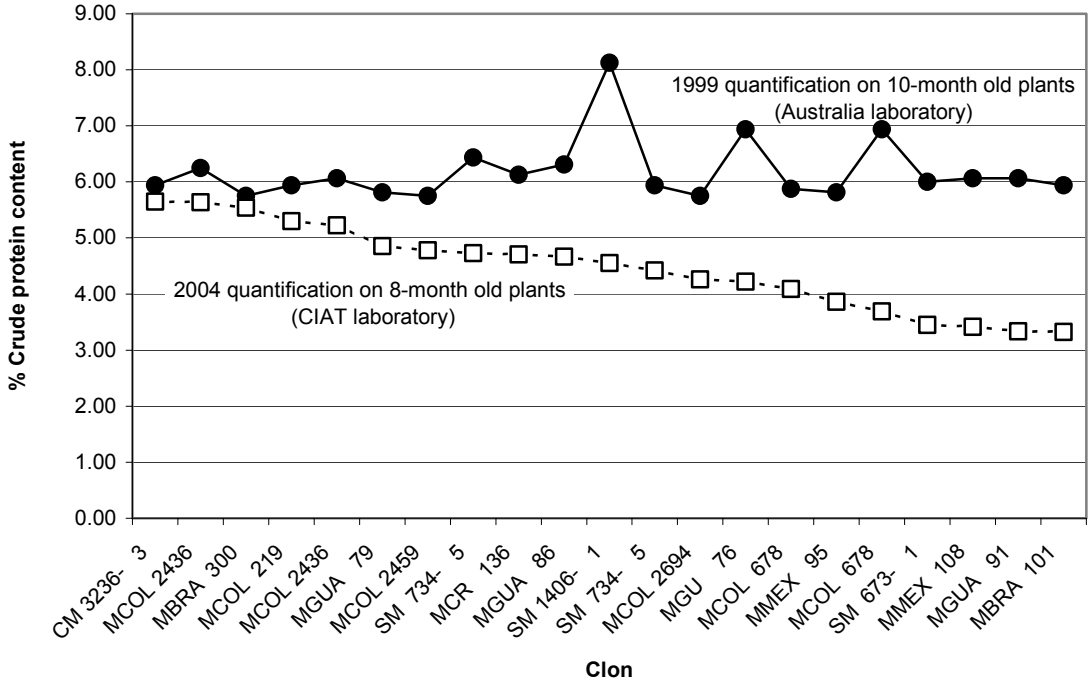

Figure 9.2. Results of crude protein content in roots from 21 cassava clones evaluated in 1999 at 10 months of age (quantification made at White's Analytical Laboratory, Adelaide University) and again in 2004 at 8 months of age (quantification at CIAT's Analytical Laboratory).

***Activity 9.3. Evaluation of* segregation of carotene content in self-pollinated progenies from selected clones.**

Rationale

In reports from previous years the results of self-pollinations of the elite clone MTAI 8 (Rayong 60) illustrated the advantages of inbreeding cassava for research purposes. One of the surprises of this early work was the apparent recessive behavior of carotene content in cassava roots. Further evaluation of self-pollinated progenies was therefore, pursued.

Specific Objectives:

a) Produce self-pollinated progenies from elite cassava clones.
b) Evaluate these progenies for carotene content in their roots.

Results.

Self-pollinated progenies from three elite clones were harvested. The most profusely sampled genotype was MTAI 8 (slightly colored roots) with a total of 181 S_1 plants, followed by yellow-

rooted clones CM 2772-3 and CM 4919-9 with only 12 S_1 plants each. At this point only ratings for color intensity can be provided because the carotene-quantification equipment if fully used for other research activities. Root samples have been stored for analysis when the analytical laboratory can proceed. However, as described in Output 1, there is an excellent correlation between color intensity and carotene content. Therefore, the results presented preliminary as they are, will suffice to illustrate the kind of segregations obtained.

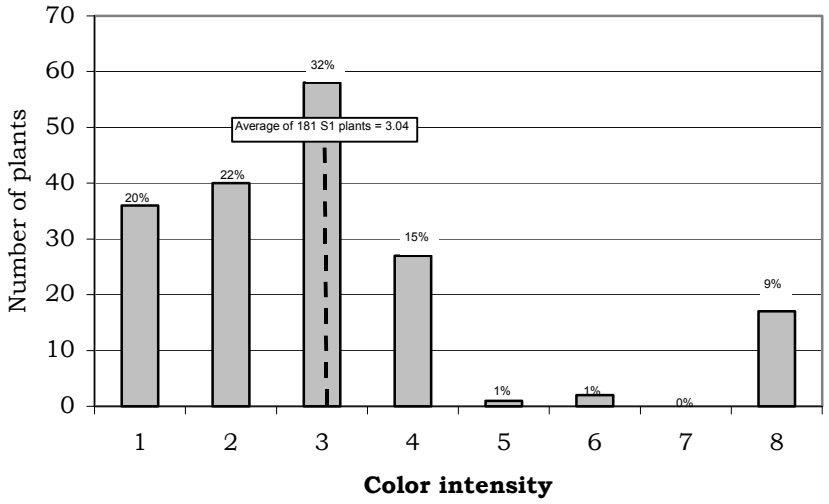

Figure 9.3. Segregations for color intensity in 181 S_1 plants from MTAI 8 (Scale 1= white; 5= deep yellow; 8= pinkish roots).

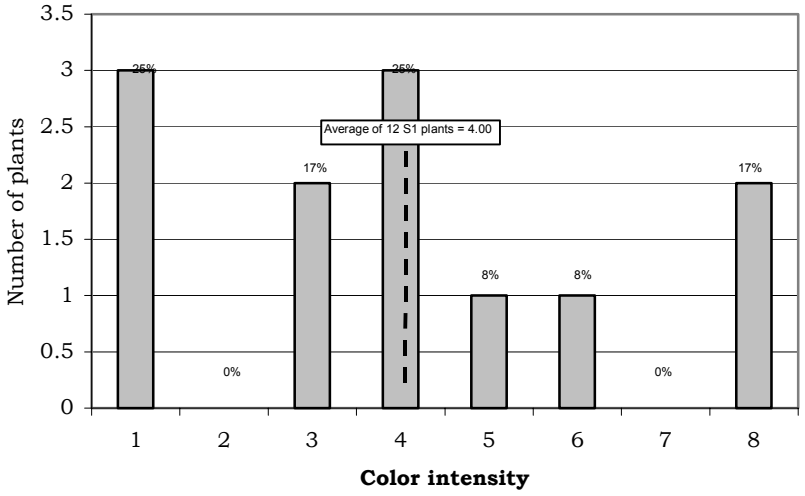

Figure 9.4. Segregations for color intensity in 12 S_1 plants from CM 4919-9 (Scale 1= white; 5= deep yellow; 8= pinkish roots).

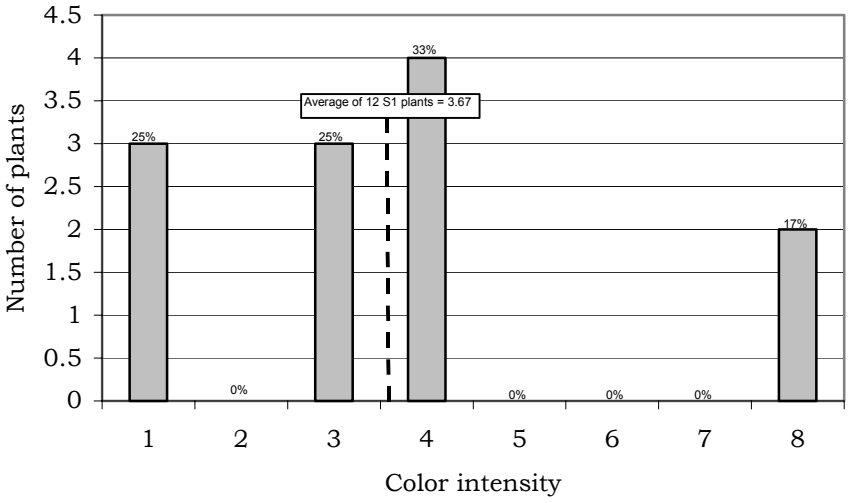

Figure 9.5. Segregations for color intensity in 12 S_1 plants from CM 2772-3 (Scale 1= white; 5= deep yellow; 8= pinkish roots).

Activity 9.4. Evaluation of segregation of traits related to Post-Harvest Physiological Deterioration.

Rationale

Post-harvest physiological deterioration (PPD) remains one major constraint for cassava development . As such the problem is dully addressed by CIAT scientists. A manuscript of a scientific article in this regard was reproduced in Output 1. This activity reports data of recently harvested clones developed to analyze the inheritance and factors affecting PPD.

Specific Objectives:

a) Produce crosses between two clones contrasting for different variables.
b) Germinate the botanical seed produced and from the resulting clones obtain stakes.
c) Plant the clones genotypes in an evaluation trial.
d) Analize the cloned genotypes for the relevant variables.

Results.

Two clones were selected for this activity. CM 523-7 (ICA-Catumare) has white roots, with high dry matter content and high susceptibility to PPD. Clon MBRA 337 has yellow roots, low dry matter content, intense yellow roots and tolerant to PPD.

A total of 38 hybrids from the cross between CM 523-7 x MBRA 337 could be cloned and planted in two replications at Santander de Quilichao (Cauca Department). Each plot had 8 plants that were harvested. The following variables will be analyzed for each plant individually: PPD, dry matter content, total starch, total sugar, reducing sugars, amylase/amylopectin ratio, cyanogenic potential. Carotenes will also be measured in pooled samples from 2-3 plants. These results will be used to learn about the individual segregations for each trait, as well as for determining the relationship among them.

Activity 9.5. Development of a quantitative genetics estimate of the standard error for test for epistasis.

Rationale

Epistasis remains one of the less understood genetic effects in plant breeding and quantitative genetics. One of the problems that make epistasis so difficult to analyze is that genetic designs for its analysis require the isolation of the within-family genetic variation, which is difficult to do. Epistasis, therefore, is frequently considered to be negligible in most quantitative genetics models. The literature seldom reports on quantitative genetics estimates of epistasis, and only occasionally the tools for determining its statistical significance is provided and used.

Specific Objectives:

a) Develop a formula for estimating the standard error of epistasis test.

Results.

In previous reports the results of diallel studies were reported. Three different diallel mating designs were used to generate F1 crosses among 9 (sub-humid and mid-altitude valleys) or 10 (acid-soil savannas) 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. Controlled pollinations were performed following the standard procedures at CIAT (Output 3). 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 a balanced 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. 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 in the respective target environments of each diallel. Table 9.1 provides the expectations for each mean square in the analysis of variance.

The analysis of variance was conducted following the expectations for each mean square described in Table 9.1. 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 σ^2_A and σ^2_D (Griffing 1956; Hallauer and Miranda 1988):

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

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

Genetic parameters were estimated using the following mean squares from Table 1:

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

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

Variance for these estimates were calculated as follows:

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

$Var\ (\sigma^2_{SCA}) = [2/(rak)^2]\quad [(MS^2_{32}\ /\ df_{32}+2) + (MS^2_{42}\ /\ df_{42}+2)]$ [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 environmental (σ^2_e) components, as illustrated in Table 1.

The within-family analysis allows obtaining information on the relative importance of epistatic effects. In the absence of epistasis the equation:

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

The variance for this test 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:

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

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

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

The last term in the equation can be estimated as:

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

in the above equation:

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

Therefore,

$$Cov(\sigma^2_{SCA}, \sigma^2_{GCA}) =$$
$$= [(1/rak) * (1/rak(p-2)] * [- Var(MS_{32}) - Var(MS_{42}) + 2 Cov(MS_{32}, MS_{42})] =$$
$$= -[2/(r^2a^2k^2(p-2)] * [(MS_{32})^2/(df+2) + MS_{42})^2/(df+2)]$$

Equation 6 can now be written as follows:

$$Var(Test) =$$
$$Var(\sigma^2_{c/F1}) + 9\,Var(\sigma^2_{SCA}) + 4\,Var(\sigma^2_{GCA}) - 12\,[2/(r^2a^2k^2(p-2)]*[(MS_{32})^2/(df+2) + MS_{42})^2/(df+2)]$$

The estimates of additive and dominance variances are overestimated because they contain portions of epistatic variances (Equations 1a and 1b).

Table 9.1. 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	
F1	[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}	$\sigma^2_e + k\,\sigma^2_\varepsilon + rk\,\sigma^2_{SCA*E} + rka\,\sigma^2_{SCA}$
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/F1	(p(p-1)/2)(k-1)	MS_6	$\sigma^2_e + r\,\sigma^2_{c/F1*E} + ra\,\sigma^2_{c/F1}$
Clones/F1*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 F1 cross (30).