Adjustment for missing plants in cassava evaluation trials

Pérez Juan C[1], Ceballos Hernan[1,2], Ramirez Isabel C[3], Lenis Jorge I.[1], Calle Fernando[1],
Morante Nelson[1].

[1] International Center for Tropical Agriculture (CIAT). Cassava Breeding Proyect. Palmira, Colombia.

[2] Universidad Nacional de Colombia – Palmira Campus, Colombia

[3] Universidad Nacional de Colombia. Medellín Campus, Colombia.

(*autor for correpondence: e-mail:j.c.pérez@cgiar.org)

Abstract

Selection in cassava (*Manihot esculenta* Crantz) breeding offers many complications. A mayor problem is the impact that storing stems has on their sprouting capacity. Evaluations with a uniform plant stand are fundamental for the reliability of their results. However, it is very difficult to correct data of missing plant within an experimental plot. Linear covariance analysis of crop yield date using plot-stand as the covariate is not a satisfactory approach especially when the plants are missed soon after sprouting or early in the growing season. The overall objective of this study was to analyze yield losses for different number of missing plants in agronomic trials and to develop a function that satisfactorily adjust plot yields at different number of missing plants. Seven clones were evaluated in different locations and for up to five years. For every variety mean plot yields decreased as the number of missing plant increased. Average losses ranged from 10.6% to 78.8% by removing one up to eight plants, respectively. Yields per plant increased significantly when more than four plants were removed; due to a compensatory growth effect. Graphic analyses showed that the power function better explained the relationship between fresh root yield and number of harvested plant. A model properly adjusted yield for all varieties but one, indicating a good fit of the proposed model. Hopefully, this formula to adjust yields can help to improve the quality of cassava trials.

Introduction

Uniform competition in cassava (*Manihot esculenta* Crantz), as for most crops, is fundamental for an acceptable magnitude of experimental errors in evaluation trials. However, it is not always feasible to achieve a perfect plant stand in cassava trials, seriously affecting their precision.

Cassava can be propagated from either stems or botanical seed, but the former is the commonest practice. The root is not a reproductive organ. Propagation from botanical seed occurs under natural conditions and is widely used in breeding programs. The morphological characteristics of cassava are highly variable. Plant height can vary from 1 to 4 m and plant type ranges from highly branching to non-branching erect types. Plant architecture influences the amount of planting material that a mother plant can produce. Erect, non-branching types generally produce larger amount of planting material and the harvest, storage and transport of stems is greatly facilitated (Ceballos and de la Cruz, 2002). The mature stem is woody, cylindrical and formed by alternating nodes and internodes. A plant grown from stem cuttings can produce as many primary stems, as there are viable buds on the cutting. However in some cultivars with strong apical dominance only one stem develops (Alves, 2001). The number of commercial stakes obtained from a single mother plant in a year ranges from three to 30, depending upon growth habit, climate, management, and soil conditions. This is considerably less than the propagation rate that can be achieved with other commercial crops that are propagated through true seed or vegetative cuttings (Leihner, 2001).

When roots are harvested the previous season, the stems are also collected and stored, typically under the shade of a tree (Ceballos et al., 2007; Morante et al., 2005). Stems can only be stored for one or two months, depending on the environmental conditions. Several factors affect sprouting capacity such as degree of lignification and thickness of the stem cutting (this means that cuttings from different parts of the stem will show differential sprouting capacity), number of nodes per cutting, varietal differences, mechanical damage of the stems (particularly of the buds), sanitary conditions of the stem regarding pest and diseases (particularly damages by stem borers) and physiological status (for example the gradual dehydration during storage affects negatively the sprouting capacity). All these factors combined with the low multiplication rate of planting material (which prevents the overplanting of evaluation plots to thin after sprouting to reduce plant densities to the adequate ones) result in frequent and chronic problems of variation in plant densities.

The effect of missing plants on plot yield may not be noticeable when there are one or two missing plants. The compensatory growth of neighboring plants usually helps to reduce differences in total plot yield. However, as the proportion of missing plants increases, the compensatory growth of the remaining plants is not enough to correct total plot yield. The effects of missing plants in total plot yields, yields per plant, and other agronomic characteristics have been investigated for different crops (Gomez and Datta,1972; James et al., 1973; Ramidi, 1995).

The covariate analysis can some times adjust cassava experimental plot yields when the plans are missing only for a short time before harvest time. However, when plants are missed throughout the growing season, competition effects and compensatory growth invalidate the linear covariance adjustment. The relationship between plot yield and plot stand is no longer linear, and an analysis of linear covariance may result in unacceptable yield estimates and failure to reduce experimental errors. The relationship between plant density and crop yield has received considerable attention (Willey & Heath, 1969; Kamidi, 1995). Most crop/yield density curves are essentially of two forms: the asymptotic response that gradually tends to an asymptote and the parabolic response that rises to a peak and then declines. In contrast, the quadratic response is symmetrical about a peak but only has flexibility in the degree of curvature so that it provides a good fit to symmetrical data but is unrealistic at low and high densities, where a gradual rise and fall would be more appropriate; it also postulates an unrealistic non-zero yield at zero density.

Different alternatives have been used to bring the yield to a comparable basis when there are one o more missing plants in an experimental plot, specially for maize (Vencovsky et al., 1991; Verones et al., 1995; Schimidt et al., 2001; Zuber, 1942). Vencovsky et al. (1991) proposed a correction based on a compensation coefficient estimated from of the experimental data, improved the adequacy of adjustments. Kamidi (1995) proposed an exponential model to correct plot stand in maize reinforcing the concept that the linear covariance analysis of crop yield data using plot stand as the covariate is no satisfactory especially when the plants are missing long before maturity.

The objectives of this work were to estimate yield losses due to missing plants in experimental agronomy cassava trials and propose a model that can be used to correct yields based on ideal plant stands.

Materials and methods

Field evaluation trials

A set of agronomic cassava experiments, with seven different varieties, were conducted during five years at four contrasting environments in Colombia (Departments of Atlántico, Cauca, Meta and Valle del Cauca). For each variety, eight different treatments were applied by removing from one, two, up to eight ‘central’ plants of each plot, as well as a control treatment (no missing plant). The plants inside experimental plots were numbered as illustrated in Figure 1, and were removed from the treatment plot to achieve the specific treatment target two month after planting (before plant competition between neighboring plants starts).

Plots consisted of five rows of five plants, spaced 1 m apart within rows and 1 m between rows (standard plant density for cassava).

The number of experiments per variety was variable (Table 1). Some varieties were evaluated only one year, while others were evaluated for the five years this study lasted and at more than one environment. Cassava varieties used were: CM 4919-9 and MTAI 8 adapted to sub-humid environment; CM 4574-7 and CM 6740-7 adapted to acid-soil savannas; CM 523-7, MCOL 1505 and SM 1058-13 adapted to the mid-altitude valleys environment. These varieties differ in branching type and, therefore, competitive ability.

The design used was a randomized complete block design with three replications per experiment. Individual analyses of variances were performed for each experiment and combined for each variety within each environment and years. Graphic analysis has been used to identify the best model to explain the relationship between fresh root yield and number of plants harvested. The information produced from these evaluation trials and analyses was used to analyze different models and select the best one based on its capacity to correct measured yields based on the ideal plants stand.

Estimation of yield losses due to missing plants

Graphic analysis was initially performed to analyze the relationship between fresh plot yields of perfect plant stands (no missing plant) versus fresh plot yield for each treatment (different number of missing plants). This graphic analysis showed that the trend explaining the relationship between fresh root yield and number of harvested plants is the power function. This function, in all cases, presented an R-square value above those of the others functions analyzed as exponential, logarithmic and lineal. The proposed model considers a power decline of yield associated with decreasing plant stand. The adjusted plot yield was, therefore, a function of both observed plot yield and plot stand, as follows:

$$y_a = y_0\left[1+\left(1-\frac{N_0}{N_a}\right)\alpha\left(\frac{N_0}{N_a}\right)^{-\beta}\right]$$

where y_a is the adjusted plot yield, y_o the observed plot yield, N_a the adjusted or planned plot stand, N_o the observed stand, and α and β are unknown parameters. This model imposes the requirement that the adjusted yield should coincide with the observed yield when planned plot stand and observed plot stand are equal. Additionally, a linear model was also fitted to the data, as follows:

$$\frac{y_a}{y_0} = \alpha + \beta\frac{N_0}{N_a}$$

where y_a, y_o, N_a, and N_o as the same mean according power model.

The α and β values were estimated by a non-liner least squares iterative procedure. The SAS non-linear regression procedure based on the modified Gauss-Newton methods was used to fit the proposed model. The models fit were assessed from the coefficients of determination (R^2) and magnitude of the residual values. Additionally, the invariances of the best models were tested. In other words this was a test to define whether or not to fit a common α and β for all cassava varieties and all environments could be found (Boche and Lavalle, 2004) statistical tests of these hypotheses were performed on the basis of the “extra sums of squares” or the conditional error principle (Milliken and Debruin, 1978).

Results

Analysis of variance for each experiment showed, as expected, significant differences among treatments for fresh root yield per plot (Table 1). The coefficient of variations (CV) ranged from 10.5% (Experiment 3) to 47.9% (Experiment 10). Experiments with CV above 30% (Exp. 10, 11 and 22) and two experiments with high root-rot incidence (Exp. 8 and 9) were eliminated from further analyses. The combined analyses of variance for each variety across years within environments showed highly significant differences among years and treatments (Table 2). The treatment-by-environment interactions were no significant, except for clone MTAI 8 that presented highly significant differences. It is important to note that varieties CM 4574-7 and CM 6740-7, adapted to the acid-soil savannas (Meta), were also evaluated in the mid-altitude valleys environment (Valle del Cauca Department).

Figure 2 illustrates the non-linear relationship between yield per plant and number of missing plants. As expected, fresh root yield on a per plant basis remains relatively stable when few plants are missing. However, as the number of missing plants is higher than 4, the yield per plant tends to increase considerably. For all varieties the mean plot yield deceased as the number of missing plant increased (Table 3). Average yield plot losses ranged from 10.6% to 78.8% by removing one up to eight plants, respectively.

The R^2 values were computed from the analysis of variance routine provided on the SAS listing. The power model was associated with a largest value of R^2 (0.9438) making it the preferred model with respect to regression model (0.5973). Convergence of power model was achieved in fewer than four interactions. Plots of the predicted yield ratio against the corresponding observed values indicated that the suggested power model was appropriate. The fitted curve and the actual values are shown in Figure 3. It can be observed that variability increases as the number of missing plants increases. In other words as number of plants increases the reliability of the adjustment is reduced.

Analysis of residuals for all analyses indicated little evidence to disprove the hypothesis that residuals were normally distributed with a mean equal to zero. The approximate F-statistics developed by Milliken and DeBruin (1978) was used to test the significance of the extra sums of squares due to common fits. Significant differences were detected between parameters for varieties and environment ($p < 0.05$). Table 4 shows the estimated parameter values individually for each variety, environment and combined data.

The fitted curves for all varieties are depicted en Figure 4. Invariance analysis for some varieties (MCOL 1505, MTAI 8 and SM 1058-13) did not show significant differences between their models indicating similar responses. Variety CM 4919-1, on the other hand, showed highly significant differences with the other varieties. Figure 5 provides the fitted curves for all environments. Valle and Cauca present a similar behavior, with smaller values, whereas Meta shows the largest predicted values. According to the information generated, therefore as expected, there was a variation in the response to missing plants for different varieties or the different environments where the trials were conducted. Nonetheless, a general model across varieties and environments was evaluated resulting in estimates for $\alpha=0.73$ and $\beta=0.81$.

The general model was used to estimates adjusted yield to uniform full plot stands for each variety (Table 3). The analysis of variance (data not shown) indicated no significant difference after adjusted yield for all varieties, except for CM 4919-1, indicating a good fit of the proposed general model to adjust yield plot when there are missing plant in experimental plots, regardless of the environment were the trials are conducted or the varieties used.

Discussion

The results obtained in this work clearly indicate the expected effects of missing plant in agronomic cassava experiments. The yield per plant increases along with the number of missing plants, mainly because the remaining plants around the missing one are favored by less competition for limiting environmental factors as light, water and fertilizer (Figure 2). The average yield when only one plant was harvested varied from 3.7 kg (CM 6740-7) to 10.2 kg (SM 1058-13), indicating large variation between varieties (Table 3).

Graphic analyses of the field data showed that the best model to explain the relationship between fresh root yield and number of harvested plant was the power function (results from different analyses not shown). For each and every analysis performed, this function presented the greatest R-square values compared with the other functions analyzed (logarithmic, exponential and linear). This model considers a power decline of plot yield as the number of missing plant increases. Additionally, the model considers that the adjusted yield should be a function of both observed plot yield and plot plant stand. The ultimate objective was to develop a model capable of adjusting total plot yields (for treatments where one or more plants were missing) as close as possible to the values observed in the perfect plant stand of the same variety. The analysis of invariance, taking into account varieties and environments, showed similar responses to different groups. However, some models showed significant differences indicting that for specific varieties and environment their parameters were different.

The general model across environments and varieties (based on α=0.73 and β=0.81) was used to adjust total plot yields as presented in Table 3. It is recognized that, ideally, the correction for missing plants should be done individually for each variety and/or location. However, the information required to make such adjustment is usually missing beforehand and, consequently, such adjustment is seldom possible. The application of a more general model that can be applied by default in the analysis of different trials would be highly desirable (Gomez and De Datta, 1972), even if the precision in the adjustment is not perfect. The interest to develop a general model applicable to different cassava varieties and environmental conditions defined the nature of this study. Different set of environments with varying average yield potential and the use of varieties with contrasting plant architectures was purposely chosen, therefore, for this study.

There are few available options to reduce the experimental errors derived from missing plants. The most obvious one would be to maximize the possibility of obtaining perfect plant stands. In many crops it is feasible to overplant and then reduce the number of surviving plants down to the desired plant density. However, in the case of cassava, availability of planting material is a chronic limitation because of the low multiplication rate (Ceballos et al., 2007). This is particularly the case in recurrent selection schemes (Morante et al. 2005). Therefore, the occurrence of missing plants is unavoidable and approaches to adjust yields a necessity. The simplest correction would be a linear approach based on the yield per plant estimate: (total plot yield/number of harvested plants)*ideal plant stand. As demonstrated (Figure 2), however, this approach would tend to overestimate corrected yields when the number of missing plants is high. Another linear correction could be based on the co-variance analysis. At the bottom of Table 3 the standard deviation of the corrected plot means for these two approaches is presented. In every case the application of the general model proposed in this study resulted in considerably smaller standard deviation values, indicating that the general model is better than other available methods.

The particular performance of cultivar CM 4919-1, a widely grown variety in the sub-humid environment of Colombia’s northern coast, was interesting because it failed to fit the general model proposed in this study. Table 5 presents information related to plant architecture of the different varieties used in this study. Plant height of CM 4919-1 was relatively low. More important, however, was that it was the only clone that did not branch at all, showing a very distinctive, completely erect plant type.

Because of the lack of a reliable method for adjusting yields in the presence of missing plants, breeders have frequently opted for two approaches, which are not satisfactory. One alternative is re-planting a cutting in the missing plant. The plant that develops from this late planted cutting is typically overcome by the plants that sprouted earlier and its yield is frequently severely reduced. The other alternative is to harvest plants in the border row (typically of the same variety) which is not satisfactory either because the plants harvested around the missing plant would have higher compensatory yields, and therefore, this approach would tend to overestimate yield potential of these plots with missing plants. The general model proposed in this study should be used in trials where no such unsatisfactory corrective measures have been used. Comparisons of coefficient of variation before and after adjusting the means would provide a fair estimate of the relative values of the method.

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Table 1. Analysis of variance per experiment for fresh root plot evalauted at four constrasting environments in Colombia.

Variety	Experiment	Mean squares			CV (%)
		Reps	Treatment	Error	
MTAI 8	1	4.454	95.817**	8.085	19.1
	2	1.449	51.048**	3.783	19.1
	3	10.490	209.155**	4.217	10.5
CM 4919-1	4	3.794	353.523**	12.525	15.7
CM 4574-7	5	2.850	108.800**	6.433	17.8
	6	3.201	44.504**	4.691	24.7
	7	3.411	41.952**	3.981	23.2
CM 6438-14	8	3.917	69.021**	3.287	18.9
	9	18.703*	17.077**	4.722	29.7
	10	49.901*	49.480**	9.434	47.9
	11	49.696*	45.299**	8.115	43.9
CM 6740-7	12	0.161	27.471**	3.806	25.6
	13	8.108	80.355**	6.291	29.7
	14	17.391	103.696**	4.959	16.2
	15	5.807	99.239**	6.785	22.0
	16	7.880	84.546**	7.604	23.0
CM 523-7	17	12.591	237.022**	18.516	21.7
	18	3.207	104.794**	4.285	13.9
MCOL 1505	19	18.918	42.059**	5.896	21.3
	20	3.009	43.242**	6.861	28.3
	21	20.867	92.671**	9.540	23.0
CM 4574-7	22	0.158	24.879	16.847	40.8
	23	13.963	122.649**	7.718	16.0
CM 6740-7	24	0.858	53.652**	7.398	25.9
	25	16.234*	51.679**	4.211	15.9
	26	12.339*	79.705**	3.378	14.1
MPER 183	27	48.517	247.664**	37.715	25.4
SM 1058-13	28	122.492	472.643**	39.864	17.7
	29	75.371*	362.933**	18.620	16.3

Table 2. Mean squares form the ANOVA for each variety cambined across years within environment.

Source of Variation	Year/ Exp	Reps	Trmnt	T x Y/ Exp	Error	CV (%)	St Error
MTAI 8 (3) (Atlántico)	600.7**	4.2	319.8**	18.1**	5.4	15.6	1.10
CM 4574-7(Meta)	274.5**	5.7	182.9**	6.2	4.8	20.8	1.03
CM 4574-7(Valle)		14.0	122.7**		7.7	16.0	2.27
CM 4574-7(All)	495.3**	4.5	287.9**	10.0	5.8	19.6	0.98
CM6740-7(Meta)	181.0**	1.2	360.3**	8.8	6.2	23.3	0.91
CM6740-7(Valle)	56.1**	3.1	161.1**	12.5	5.4	19.2	1.01
CM6740-7(All)	133.9**	1.2	505.8**	10.8	5.9	21.6	0.71
MCOL1505(Valle)	138.9**	14.0	152.7**	7.3	6.7	22.5	1.22
CM523-7(Valle)	335.0**	2.0	322.7**	19.1	11.5	19.6	1.96
SM1058-13(Cauca)	837.8**	19.4	861.0**	59.1	31.8	18.4	3.26
CM4919-1(Atlántico)		3.8	353.5**		12.5	15.7	2.89

Table 3. Varieties mean of observed plot yield and adjusted plot yield.

Harvested plants	MTAI 8		CM 4919-1		CM 4574-7		CM 6740-7		CM 523-7		MCOL 1505		SM 1058-13	
	y_o	y_a	y_o	y_a	y_o	y_a	y_o	y_a	y_o	y_a	y_o	y_a	y_o	y_a
9	22.7	22.7	39.1	39.1	18.9	18.9	17.3	17.3	25.5	25.5	16.8	16.8	45.2	45.2
8	19.5	21.2	32.8	35.7	16.4	17.8	15.9	17.3	22.8	24.8	15.3	16.6	44.4	48.4
7	20.3	24.4	31.1	37.3	15.4	18.5	14.4	17.3	22.4	26.9	14.7	17.7	37.1	44.4
6	16.7	22.3	26.0	34.7	13.6	18.1	13.7	18.3	24.9	33.3	13.6	18.1	37.0	49.5
5	17.2	26.2	21.9	33.3	15.2	23.1	12.2	18.5	16.6	25.1	12.0	18.3	33.6	51.0
4	13.5	24.0	19.5	34.6	11.1	19.8	8.9	15.8	17.6	31.3	10.3	18.3	28.8	51.5
3	11.4	24.8	16.9	36.7	9.9	21.4	8.8	19.2	12.0	26.0	9.4	20.3	22.4	48.6
2	8.2	23.8	10.2	29.4	5.8	16.8	6.3	18.1	9.3	26.8	7.0	20.2	17.8	51.5
1	4.7	22.2	5.7	27.3	4.7	22.5	3.7	17.7	4.8	22.9	4.3	20.4	10.2	48.5
St. Dv.[1]	**5.96**	**1.54**	**10.86**	**3.78**	**4.83**	**2.20**	**4.59**	**0.96**	**7.33**	**3.30**	**4.12**	**1.46**	**11.98**	**2.50**
St.Dev.[2]	**6.79**	**9.69**	**5.39**	**13.45**	**7.58**	**8.13**	**5.49**	**6.48**	**6.88**	**12.93**	**7.29**	**5.40**	**15.48**	**18.77**

y_o= observed plot yield, y_a= adjusted plot yield.

[1] For each variety, standard deviations for observed plot yields (left) and using the general model correction (right).

[2] For each variety, standard deviations for observed plot yields corrected by the yield per plant approach (left) or the linear approach (right)

Table 4. Estimated parameter values for varieties, environment and for combined data.

Variety	**Alpha**	**Beta**
CM 4574-7	1.052 (0.1956)	0.579(0.0985)
CM 4919-1	0.870(0.0836)	0.920(0.0477)
CM 523-7	0.462(0.1122)	1.070(0.1183)
CM6740-7	0.821(0.1761)	0.774(0.1183)
MCOL 1505	0.537(0.1786)	0.840(0.1673)
MTAI8	0.550(0.1293)	0.934(0.1165)
SM 1058-13	0.548(0.1177)	0.901(0.1070)
Average	**0.691**	**0.718**
Environment		
Cauca	0.548(0.1177)	0.901(0.1070)
Atlántico	0.630(0.1449)	0.929(0.1114)
Meta	1.1591(0.1747)	0.6415(0.0787)
Valle	0.455(0.0882)	0.948(0.0958)
Average	**0.698**	**0.855**
Common fit (all experiments)	**0.727(0.0805)**	**0.805(0.0559)**

Table 5. Averages of relevant plant type characteristics of the varieties used in this study.

Genotype	Height (cm)		Number of
	Plant	1st branching	branching events
MTAI 8	200	120	3
CM 4919-1	205	-.-	-.-
CM 4574-7	245	175	2
CM 6740-7	297	167	2
CM 523-7	220	115	3
MCOL 1505	215	100	3
SM 1058-13	200	56	5

0	0	0	0	0
0	1	2	3	0
0	4	5	6	0
0	7	8	9	0
0	0	0	0	0

Figure 1. Scheme illustrating the identification of each ‘central’ plant inside experimental plots for measuring the effect the missing plant in cassava evaluation trials.

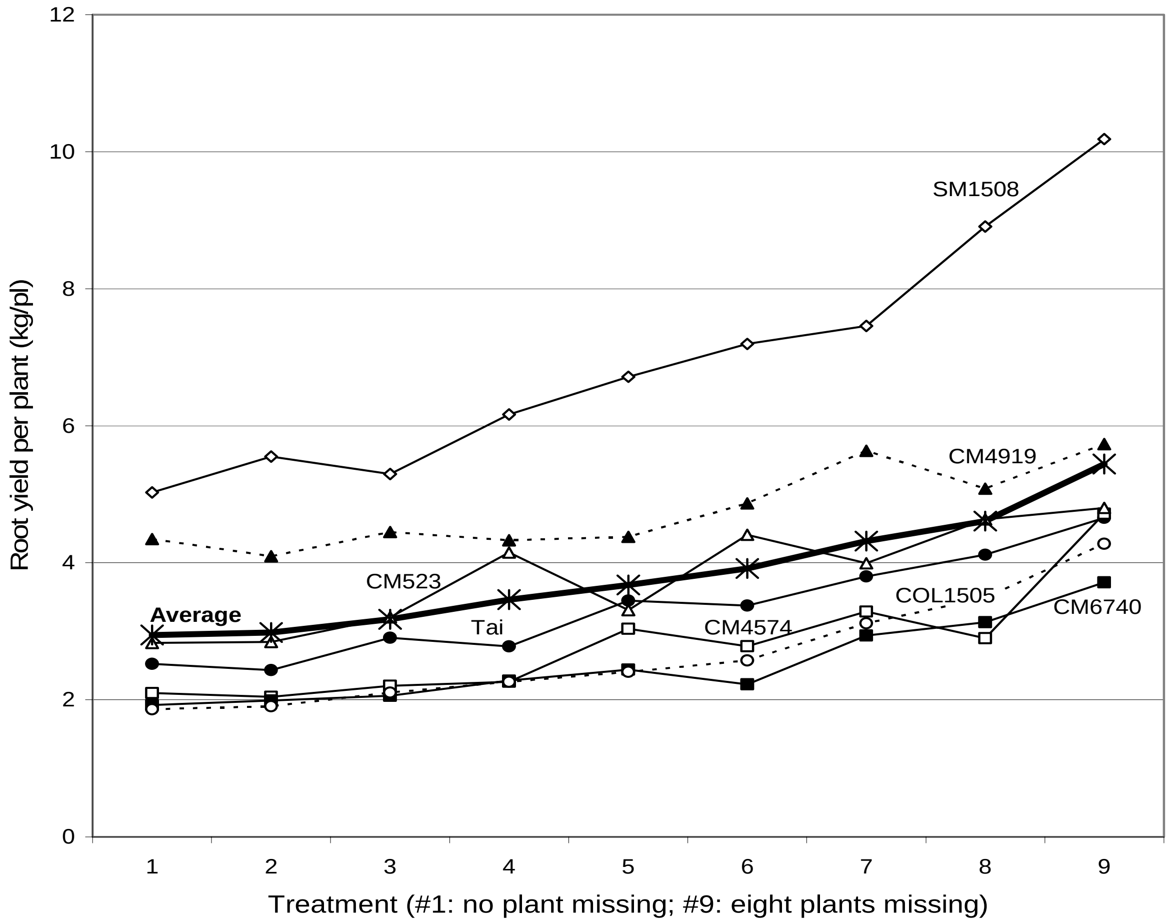

Figure 2. Yield per plant of different varieties (across trials). The non-linear relationship becomes evident when yield per plant increases as the number of missing plants is higher than 4.

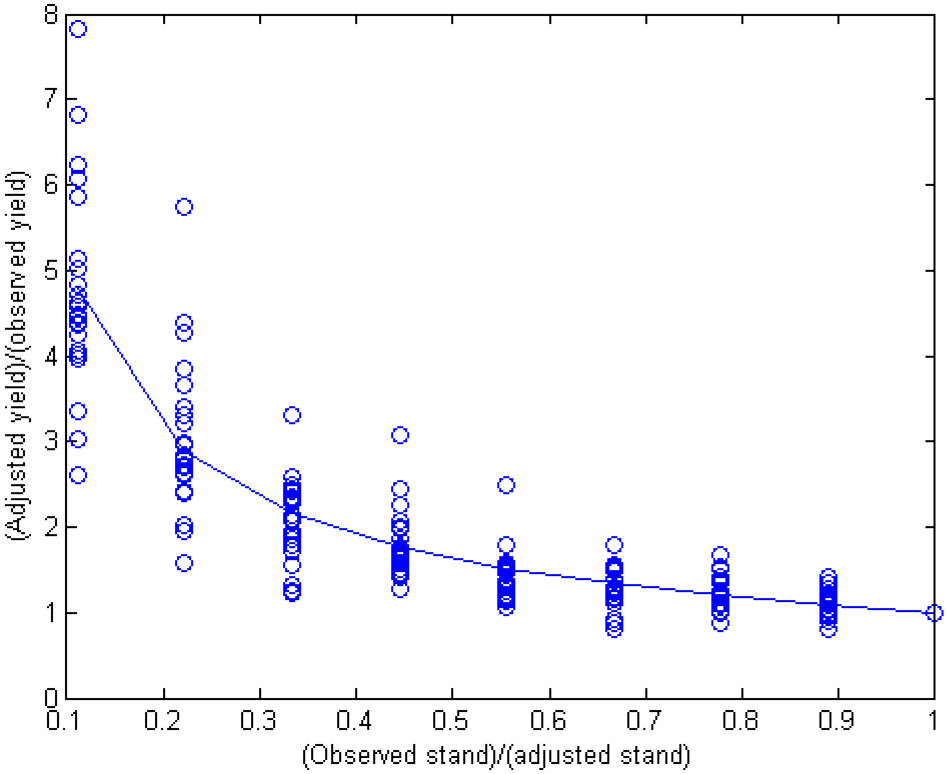

Figure 3. Prediction curve for general values

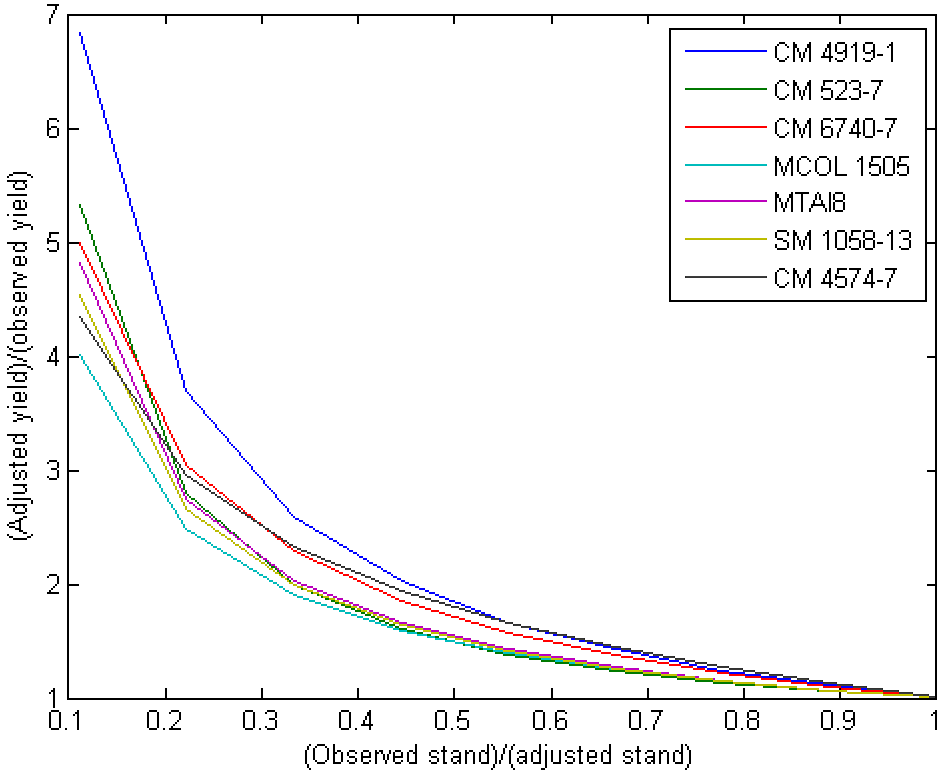

Figure 4. Prediction curve for all varieties

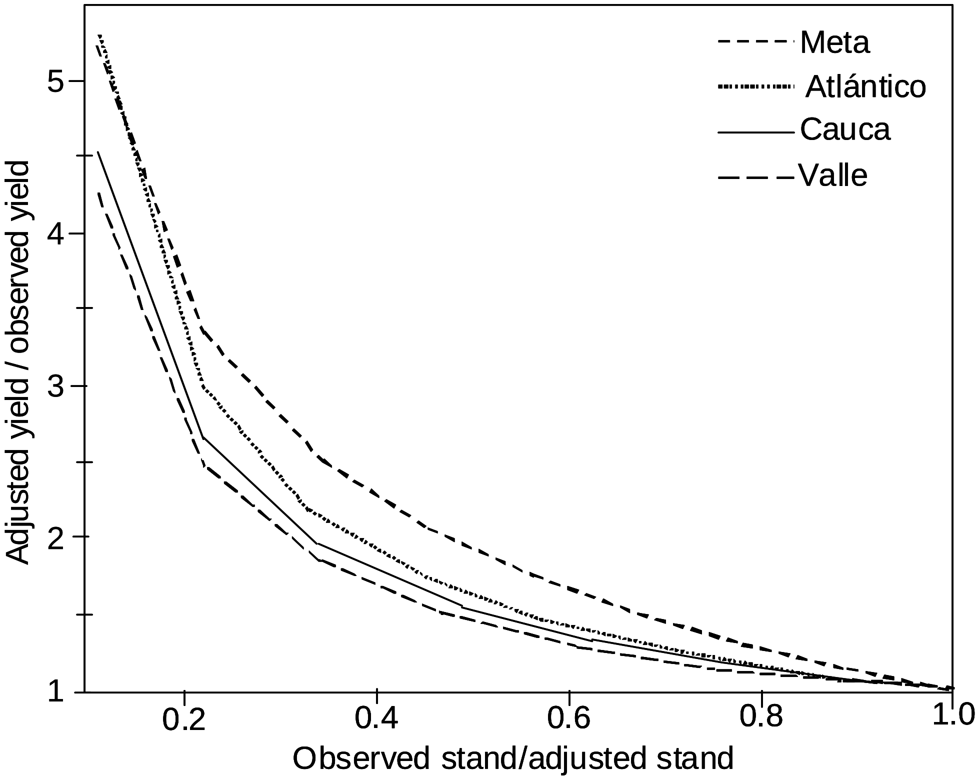

Figure 5. Prediction curve for all environments