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Table of Contents: Volume 9 Number 2 February, 2017

ARTICLES

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Full Length Research Paper

Estimates of combining ability and heritability in cowpea genotypes under drought stress and non-stress conditions in Uganda

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Cowpea is an important source of food and income for small scale farmers in Uganda. Production is, however, affected by both biotic and abiotic stresses. Drought stress has recently emerged as a serious concern due to the effects of climate change. This study was therefore undertaken to estimate the general and specific combining ability effects of parents and crosses as well as estimate the heritability of delayed leaf senescence, seed yield and its components under drought stress. Five drought tolerant genotypes were crossed with four drought sensitive genotypes in a North Carolina II mating design. The study revealed that drought tolerance is conditioned by both additive and non-additive genetic effects with the predominance of non-additive genetic effects for seed yield, 100 seed weight and number of pods per plant. Delayed leaf senescence was however, controlled by additive genetic effects, implying that progenies performance could be predicted from parents General Combining Ability (GCA) effects. The cultivars SECOW 5T, IT93K-452-1 and IT98K-205-8 were good combiners for drought tolerance. The F2 families: SECOW 3B x IT98K-205-8, SECOW 5T x IT98K-205-8, SECOW 4W x IT98K-205-8 and SECOW 1T x IT98K-205-8 had positive Specific Combining Ability(SCA) effects in seed yield, number of pods per plant and 100 seed weight, implying that they performed better than what was predicted by their parents GCA effect. As such, they are promising cross combinations that can be advanced for later generation selection.

Key words: Drought stress, combining ability, water use efficiency.

INTRODUCTION

Drought remains a challenge in the eastern and north eastern regions of Uganda where cowpea is predominantly grown. These areas are already grappling with high water stress, rapid population growth, environmental degradation, and low socio-economic growth (Bigirimana, 2011). Cultivation of plants that are adapted to water

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stress conditions is necessary in order to reduce crop production losses and meet the need for food for the growing population (Fageria et al., 2007). Breeding for drought tolerance lessens the danger of crop failure by improving the crop's ability to extract water from the soil, improving its water use efficiency and ability to survive longer periods without water. Breeding for drought tolerance, however, requires knowledge of the inheritance and genetic variability for traits conferring drought tolerance (Chiulele, 2010). This information is required for the identification of the best parents and selection strategies to use in breeding. In light of this, two approaches have been proposed for screening and breeding for drought tolerance in cowpea. These include: One that uses grain yield and its components, as the primary criteria and one that characterizes specific morpho-physiological traits that contribute to growth and yield in the event of drought (Agbicodo et al., 2009).

Cowpea exhibits genetic variability for drought tolerance (Muchero et al., 2008). Gene action studies conducted elsewhere have reported the predominance of additive genetic effects over non-additive genetic effects in controlling yield components such as days to flowering, number of seeds per pod, number of pods per plant and hundred seed weight (Chiulele, 2010; Romanus, 2008). Non-additive genetic effects were, however, more important for seed yield (Chiulele, 2010; Alidu et al., 2013). Thus, information regarding combining ability and nature of gene action governing the inheritance of desirable traits are basic requirements for breeding high yielding drought tolerant cowpea genotypes. However, such information is not available for cowpea genotypes in Uganda. Combining ability and heritability estimates are specific to germplasm being tested and the testing environment (Falconer, 1989). Knowledge of the genetic control of complex quantitative traits and the magnitude of genetic variability that exists among the available germplasm are important for selection and genetic improvement of the crop (Umar et al., 2014). Selection of parental genotypes based on combining ability estimates has been used as an important breeding approach in crop improvement (Umar et al., 2014). This study was, therefore, carried out in order to estimate the general and specific combining ability effects of parents and crosses as well as estimate the heritability of delayed leaf senescence, seed yield and its components. The information generated will help in planning and implementation of an efficient breeding program for improvement of drought tolerance levels of cowpea in Uganda.

MATERIALS AND METHODS

Study area

The study was conducted in a water proof screen house at the Makerere University Agricultural Research Institute - Kabanyolo (MUARIK). MUARIK is located at an altitude of 1217 m above sea level on coordinates 0.16° 24' 16 N and 32.5° 27' 34 E, approximately 19 km northeast of Kampala within the Lake Victoria Crescent. The average temperature and relative humidity in the screen house during the study ranged from 25 to 38°C and 70 to 92%, respectively.

Developing a breeding population

Four confirmed exotic drought tolerant lines from IITA, Kano (IT 98K-205-8, IT99K-573-1, IT 93K-452-1, IT 89KD-288) and five released Ugandan lines (SECOW 1T, SECOW 2W, SECOW 4W, SECOW 3B, SECOW 5T) with desirable disease resistance characteristics were crossed to generate a total of 20 F_1 families. The nine parents were crossed in the screen house using a North Carolina II mating design (Acquaah, 2007). The NC II mating design is utilized in general and specific combining ability estimation and in determining the relative importance of additive over non- additive genetic effects (Acquaah, 2007).

Population advancement and drought tolerance screening

The F_1 plants of each of the 20 F_1 crosses were selfed to generate F_2 seeds. However, one cross involving SECOW 2W with IT 99K-573-1 did not germinate possibly due to seed dormancy attributed to both the embryo and seed coat factors. The F_2 seeds together with the nine parents and seven checks were planted in 10 L plastic pots and the plants were phenotypically characterized for drought tolerance for the following traits: Delayed leaf senescence, number of pods per plant, number of seeds per pod, 100 seed weight and seed yield. The genotypes were subjected to drought stress after the emergence of the flower bud on individual pot basis. The experiment was laid out in a randomized complete block design in a split plot arrangement with five replications and two watering regimes. The watering regime was the main plot and test genotypes, each represented by four plants per water regime, formed the sub plots. The watering regimes were: T1, as no stress (NS) and T2, as severe water stress (DS) treatments. Plants in the well-watered treatment (no stress) were maintained at field capacity (50% soil water capacity) throughout the experimental period by measuring the soil moisture content using an MO750 soil moisture meter twice a week and applying water to restore the appropriate moisture level. The severe water stress treatment did not receive water for 20 days, then it was re-watered.

Data collection

Data was collected on individual plant basis in line with the international plant genetic resources cowpea descriptors; days to 50% flowering and delayed leaf senescence. Plants were scored for leaf senescence on a scale of 1 to 5, with 1 = totally green and turgescent, 2 = green and slightly wilted, 3 = green yellow and wilt, 4 = yellow-green and severely wilt and 5 = completely yellow to brown / almost died (Muchero et al., 2008). At maturity, the plants were harvested to determine yield components: number of pods per plant, number of seeds per pod, 100 seed weight and seed weight per plant.

Data analyses

The data collected were analysed using Genstat software 12th edition of VSN International. The means of the 19 F_2 family crosses, seven checks and nine parents were compared in an analysis of variance using the following linear model: The Linear Mathematical

Table 1. Mean squares for response of 19 F_2 population, 7 checks and 9 parents to drought stress induced at the reproductive stage.

Source of variation	DF	NPP	NSP	100 SWT	Seed Yield
Replications	4	17.22	28.42	26.17	113.77
Watering regime	1	100.54***	945.89***	1633.03***	2879.56***
Main Plot Error	4	2.74	11.18	9.54	34.23
Genotypes	34	7.42***	18.91***	22.94***	61.42***
Genotypes * Water level	34	3.53	9.39	8.31*	27.37**
Sub Plot Error	269	2.43	8.38	5.54	14.16

*, **, *** significant at P ≤ 0.05, 0.01 0.001 respectively; DF, degrees of freedom; NPP, number of pods per plant; NSP, number of seeds per pod; 100SWT, 100 seed weight. MP Error, Main plot error; SP Error, Subplot error.

Model for split plot experimental design used was as follows:

$X_{ijk}=Y... +M_i +B_j +d_{ij}+ S_k+ (MS)_{jk}+ e_{ijk}$

Where X_{ijk} = mean observations, Y= the experiment mean, M_i = the main plot treatment effect, B_j = replication or block effect, d_{ij} = the main plot error (error a), S_k = the subplot treatment effect, $(MS)_{ik}$ = the main plot and subplot treatment interaction effect, e_{ijk} = the subplot error (error b). i = a particular main plot treatment, j = a particular block, k = a particular subplot treatment. Combining ability analysis was performed to compare the means of the 19 F_2 family crosses. The genetic variance component was partitioned into general combining ability (GCA) and the specific combining ability (SCA) variances according to Dabholkar (1992). The linear model used was:

$Y_{ijk} = U + f_j + m_k + (fxm)_{jk} + e_{ijk}$

Where: Y_{ijk} = effects observed due to r^{th} replications, j^{th} female and k^{th} male; U = Overall mean of the experiment; r_i = Observed effects due to i^{th} replication; f_j = GCA effects due to the j^{th} female parent; m_k = GCA effects due to the k^{th} male parent; $(fm)_{jk}$ = SCA effect due to the Interaction between k^{th} male and j^{th} female; and e_{ijk} = Random error of the experiments. Broad and narrow sense coefficients of genetic determination (BSCGD and NSCGD) were estimated on genotype mean basis following the formulas outlined by Ruming, 2004 (Ozimati et al., 2014) as follows:

$$\text{BSCGD} = \frac{\sigma^2\text{GCAf} + \sigma^2\text{GCAm} + \sigma^2\text{SCAfm}}{\sigma^2\text{GCAf} + \sigma^2\text{GCAm} + \sigma^2\text{SCAfm} + \sigma^2 e/_r}$$

$$\text{NSCGD} = \frac{\sigma^2\text{GCAf} + \sigma^2\text{GCAm}}{\sigma^2\text{GCAf} + \sigma^2\text{GCAm} + \sigma^2\text{SCAfm} + \sigma^2 e/_r}$$

$$\text{Bakers' ratio (BR)} = \frac{\sigma^2\text{GCAf} + \sigma^2\text{GCAm}}{\sigma^2\text{GCAf} + \sigma^2\text{GCAm} + \sigma^2\text{SCAfm}}$$

Where, δ^2GCA_f = variance component general combining ability for female parents, δ^2GCA_m = variance component general combining ability for male parents, δ^2SCA_{fm} = variance component specific combining ability for the hybrids, δ^2e = variance component error, r = number of replications. The variance component for males were calculated by subtracting their mean squares from the error mean squares and dividing the males by the number of females involved in the crossing vice-versa (Dabholkar, 1992). To quantify drought severity, the drought intensity index (DII) was calculated using the formula suggested by Fischer and Maurer (1978) as follows: DII = 1 - Xs/Xp, where Xs and Xp are the mean grain yield of all the genotypes of the same maturity group under drought stress (DS) and non-stress(NS) conditions, respectively.

RESULTS

Response of F_2 population and parental genotypes to drought stress

The analysis of variance for seed yield, number of pods per plant, number of seeds per pod and 100 seed weight for parents, checks and F_2 population showed that the parents, checks and F_2 population were significantly different (P ≤ 0.001). The watering regimes explained much of the variation that was seen in seed yield and its components as indicated by the high mean squares (Table 1). The mean performance of all the crosses in seed yield, number of seeds per pod, 100 seed weight was higher than the average performance of all the parents except for delayed leaf senescence (Table 2).

Combining ability analysis

Mean squares of crosses (Table 3) were significant under water stress for delayed leaf senescence (P ≤ 0.05), number of pods per plant (P ≤ 0.01), 100 seed weight (P ≤ 0.01) and seed yield (P ≤ 0.001). Further partitioning of variance of crosses into that due to male and female parents attributed to GCA, and that due to male and female interaction attributed to SCA showed that under water stress conditions, the mean squares for general combining ability (GCA) for male parents (Table 3) were significantly different for number of pods per plant, number of seeds per pod (P ≤ 0.01), 100 seed weight (P ≤ 0.01) and seed yield (P ≤ 0.001). The mean squares for GCA for female parents (Table 3) were only significantly different for delayed leaf senescence (P ≤ 0.05). The mean squares for the specific combining ability (SCA) of parental combinations were significantly different for number of pods per plant (P ≤ 0.01), 100 seed weight (P ≤ 0.05) and seed yield (P ≤ 0.001). The results also showed that in the absence of water stress, the mean squares for GCA for male parents were only significantly different (P ≤ 0.01) for 100 seed weight and seed yield (Table 3).

Table 2. Means of seed yield and its components in F_2 populations and their parents.

Population	Genotype	NS NSP	DS NSP	NS NPP	DS NPP	NS 100 SWT	DS 100 SWT	NS Seed yield	DS Seed yield	DS DLS
	SECOW 2W X IT 93K-452-1	10.6	6.61	4.2	5	9.35	4.96	14.02	11.29	66.33
	SECOW 4W X IT 93K-452-1	8	8.41	4.6	2.4	9.19	2.41	13.79	4.12	69.14
	SECOW 3B X IT 93K-452-1	10.41	6.52	4.66	4.23	6.85	4.22	10.28	7.06	37.77
	SECOW 5T X IT 93K-452-1	11.2	8.01	3.6	2.8	9.77	2.25	14.65	3.07	71.83
	SECOW 1T X IT 93K-452-1	12	7.41	4.2	2.6	8.39	1.93	12.59	4.33	74.99
	SECOW 2W X IT 98K-205-8	9.2	1.81	4	1.4	5	0.36	7.5	0.56	58.33
	SECOW 4W X IT 98K-205-8	10.4	4.81	5.2	3	7.09	2.97	10.63	4.83	73.99
	SECOW 3B X IT 98K-205-8	10.6	6.41	3.8	3.4	8.07	4.31	12.11	8.41	54.33
F_2	SECOW 5T X IT 98K-205-8	9	6.81	3.8	4.2	6.52	3.34	9.77	5.51	76.99
	SECOW 1T X IT 98K-205-8	8.4	5.61	3.6	3.8	7.13	2.48	10.69	2.99	70.59
	SECOW 4W X IT99K-573-1	10.01	6.61	3.96	2	5.2	1.34	7.8	2.21	68.33
	SECOW 3B X IT99K-573-1	7.6	5.01	3.4	2.2	5.05	1.3	7.58	1.73	68.33
	SECOW 5T X IT99K-573-1	8.4	6.81	3.8	2.6	6.82	2.82	8.63	4.32	80.99
	SECOW 1T X IT99K-573-1	10.4	3.81	3.6	2.2	6.63	1.19	9.94	2.01	80.99
	SECOW 2W X IT 89KD-288	10.4	5.21	3.4	3.2	3.43	2.1	5.15	2.74	84.25
	SECOW 4W X IT 89KD-288	8.4	5.61	4.8	2.8	7.15	1.72	10.73	2.78	67.14
	SECOW 3B X IT 89KD-288	8	4.01	2.6	3.8	4.38	2.24	6.56	4.33	63.61
	SECOW 5T X IT 89KD-288	10	3.81	5.2	1.6	6.87	0.73	10.3	0.73	82.42
	SECOW 1T X IT 89KD-288	10.8	5.01	3.4	2.2	9.49	1.33	14.23	1.85	59.14
Mean		9.67	5.7	3.99	2.92	6.97	2.32	10.37	3.94	68.92
	SECOW 2W	9.8	5.81	3.8	1.8	2.56	1.39	2.82	3.17	79.99
	SECOW 4W	6.6	3.61	2.4	2	3.09	0.73	4.64	1.63	94.99
	SECOW 5T	9	4.41	3	0.8	2.33	1.2	3.23	2.01	75.6
	SECOW 1T	11	5.21	2.8	1.8	6.48	0.75	8.21	1.16	83.09
Parents	SECOW 3B	8.6	6.01	3.6	3.4	6.17	1.53	7.84	2.01	60.16
	IT98K-205-8	5.6	5.21	5.8	3.6	4.8	1.88	7.2	6.47	62.49
	IT99K-573-1	4.8	1.61	2.6	0.8	1.47	0.18	2.2	4.07	84.66
	IT93K-452-1	7	6.21	6.4	5.4	8.98	3.39	13.46	7.44	64.99
	IT89KD -288	6.6	4.41	5.6	3.8	7.63	2.61	11.45	4.73	73.33
Mean		7.67	4.72	4	2.6	4.83	1.52	6.78	3.63	75.48
LSD (5%)		3.2	3.73	2.1	1.69	3.5	2.21	5.4	3.85	24.2

The estimates of narrow sense coefficient of genetic determination was very low (0.01-0.05) for number of pods per plant and number of seeds per pod. The estimates of broad sense coefficient of genetic determination were moderately high to high (0.68-0.8) for number of pods per plant, 100

Table 3. Mean squares, variance components, Bakers' ratio and coefficient of genetic determination on entry mean basis for the response of 18 F_2 population to drought stress.

Source of variation	df	No stress					Drought stress			
		NPP	NSP	100 SWT	Seed yield	DLS	NPP	NSP	100 SWT	Seed yield
GCA $_{female}$	4	0.67	1.14	2.79	5.76	257.44*	0.5	1.98	0.9	4.53
GCA $_{male}$	3	0.3	1.39	8.30**	20.83**	106.5	1.07*	7.19**	2.75**	12.76***
SCA $_{(female.male)}$	11	0.4	1.8	2.03	4.46	84.44	1.03**	1.69	1.42*	6.66***
Crosses	18	0.45	1.58	3.25*	7.48*	126.56*	0.92**	2.67	1.53**	7.20***
Error	133	0.55	1.28	1.6	3.74	73.9	0.36	1.78	0.62	1.9
Variance components										
GCA $_{female}$	4	0.03	-0.04	0.30	0.50	45.89	0.03	0.05	0.07	0.66
GCA $_{male}$	3	-0.05	0.02	1.34	3.42	6.52	0.14	1.08	0.43	2.17
SCA $_{(female.male)}$	11	-0.14	0.52	0.43	0.71	10.54	0.66	-0.09	0.80	4.76
Coefficient of genetic determination on entry mean basis										
Bakers ratio		1	0.04	0.79	0.85	0.83	0.21	1	0.38	0.37
BSCGD		0.05	0.3	0.56	0.55	0.46	0.70	0.39	0.68	0.80
NSCGD		0.05	0.01	0.45	0.47	0.38	0.15	0.39	0.26	0.30

*, **, *** significant at P ≤ 0.05, 0.01 0.001 respectively, NPP, number of pods per plant; NSP, number of seeds per pod; 100SWT, 100 seed weight; DLS, delayed leaf senescence; GCA $_{FEMALE}$, General combining ability for female parents; GCA $_{MALE,}$ General combining ability for male parents; SCA $_{(FEMALE.MALE),}$ Specific combining ability for male and female combinations; BSCGD, Broad sense coefficient of genetic determination; NSCGD, Narrow sense coefficient of genetic determination. NB, The negative variance components obtained for number of seeds per pod under no water stress and under stress could not be used to estimate heritability; and were treated as zero. The negative values of these variance components were attributed to experimental error.

seed weight and seed yield but moderate (0.39-0.46) for delayed leaf senescence and number of seeds per pod. The estimates of narrow sense coefficient of genetic determination was low to moderate (0.15-0.39) for all the traits.

Estimates of general and specific combining ability effects

Female parent SECOW 5T showed desirable significant positive GCA effect for seed yield (Table 4). SECOW 2W showed average positive GCA effects for number of pods per plant, 100 seed weight and average GCA effects for seed yield and number of seeds per pod. However, SECOW 2W showed undesirable significant positive GCA effect for delayed leaf senescence. SECOW 4W showed desirable significant positive GCA effect for number of seeds per pod and average effects for number of pods per plant, 100 seed weight, seed yield and a negative average GCA effect for delayed leaf senescence. SECOW 3B and SECOW 1T showed undesirable significant negative GCA effects for number of pods, 100 seed weight and seed yield. However, SECOW 1T showed the highest desirable significant negative GCA effect for delayed leaf senescence. The male parent IT 93K-452-1 and IT98K-205-8 showed desirable significant positive GCA effects for seed yield, number of pods per plant, number of seeds per pod, 100 seed weight and a desirable average GCA effect and significant negative GCA effect for delayed leaf senescence respectively (Table 4). Hence, they were good combiners. IT99K-573-1 showed undesirable significant negative GCA effects for seed yield, number of pods per plant, number of seeds per pod, 100 seed weight. IT99K-573-1 also showed a positive undesirable GCA effect on delayed leaf senescence, hence, it was a poor combiner.

SECOW 5T x IT98K-205-8 and SECOW 1T x IT98K-205-8 showed desirable significant positive Specific combining ability effects for seed yield, number of pods per plant, number of seeds per pod, 100 seed weight and a desirable significant negative SCA effects for delayed leaf senescence (Table 5). SECOW 4W x IT98K-205-8 showed

Table 4. Estimates of general combining ability effects of seed yield and its components for male and female parents.

Male parents	No stress					Stressed			
	NPP	NSP	100 SWT	Seed yield	DLS	NPP	NSP	100SWT	Seed yield
IT98K-205-8	0.06	-0.85	-0.54	-0.81	-15.38***	1.46***	3.08***	1.84***	3.31***
IT99K-573-1	0.26	-0.05	-1.44*	-2.16*	15.26***	-1.26***	1.32***	-1.03**	-3.73***
IT93K-452-1	0.26	0.55	3.80***	5.71***	-7.38	2.14***	1.72***	2.76***	7.42***
IT89KD-288	-0.54	0.35	-2.11***	-3.17***	10.54**	0.34	0.32	-0.09	-1.13
	No stress					Stressed			
Female parents	NPP	NSP	100 SWT	Seed yield	DLS	NPP	NSP	100 SWT	Seed yield
SECOW 4W	0.89*	-1.07	0.74	1.11	-3.49	0.11	0.91**	0.12	0.31
SECOW 3B	1.29***	0.53	0.46	0.68	11.79**	-1.09***	-0.89**	-0.87*	-1.74*
SECOW 1T	-0.51	1.33*	3.08***	4.61***	-11.49**	-0.49	0.31	-0.27	-0.63
SECOW 5T	-1.31***	-1.47*	-2.04**	-3.05**	-7.02	1.11***	-0.69*	0.64	1.86**
SECOW 2W	-0.51	0.93	-2.98***	-4.47***	13.62**	0.51	0.51	0.50	0.27

*, **, *** significant at P ≤ 0.05, 0.01 0.001 respectively, NPP, number of pods per plant; NSP, number of seeds per pod;100SWT, 100 seed weight; DLS, delayed leaf senescence.

desirable positive SCA effects for yield and its components except for number of seeds per pod and a desirable significant negative SCA effects for delayed leaf senescence while SECOW 3B x IT98K-205-8 showed desirable SCA effects for yield and its components and an average negative SCA effect for delayed leaf senescence. The crosses SECOW 3B x IT 93K-452-1, SECOW 3B x IT99K-573-1 and SECOW 3B x IT89KD-288 showed desirable negative SCA effects for delayed leaf senescence. The cross SECOW 1T x IT93K-452-1 showed undesirable positive SCA effect on delayed leaf senescence (Stay green trait). SECOW 5T x IT93K-452-1 exhibited a significant non-desirable negative SCA effect for seed yield (Table 5).

DISCUSSION

Genetic variation

The overall goal for all plant breeding programs is achieving yield gains and this is dependent on having genetic diversity for the trait under selection with a higher heritability (Falconer and Mackay, 1996). The analysis of variance showed that genotypes were significantly different in most traits evaluated under water deficit conditions, an indication of the existence of genetic variability among parents and their progenies for drought tolerance, favoring selection. The mean performance of most crosses in seed yield, number of seeds per pod, 100 seed weight was higher than most parental genotypes except for delayed leaf senescence, an indication of the presence of transgressive segregants (Table 2), which may have been as a result of recombination of additive alleles (complementary gene action) or interaction between two alleles of two different genes due to a wider genetic distance between genotypes of their parents (Marame et al., 2009). The presence of transgressive segregants suggests polygenic inheritance and breeding strategies such as backcrossing, multiple crossing, heterosis and pedigree breeding methods with recurrent selection could facilitate the simultaneous exploitation of these favorable alleles.

The average seed yield, number of pods per plant, number of seeds per pod and 100 seed weight was higher under no moisture stress than under moisture stress conditions for both parents and progenies (Table 2), indicating that moisture stress significantly reduced seed yield and its components of the parents and progenies. The intensity of the drought stress as shown by the drought Intensity Index value of 0.56 was sufficient to reduce the yield and its components of the cowpea genotypes. DII values above 0.7 are considered as severe drought stress (Ramirez-Vallejo and Kelly, 1998). Shirinzadeh et al. (2010) selected Maize and dry bean cultivars as sources of drought tolerance in the field with DII values ranging between 0.51 and 0.69, so the drought stress intensity in this study was sufficient to separate drought tolerant cultivars from drought

Table 5. Estimates of specific combining ability effects for the crosses under water stress.

F2 Population	NS	DS	NS	DS	NS	DS	NS	DS	DS
	NPP	NPP	NSP	NSP	100 SWT	100 SWT	Seed Yield	Seed Yield	DLS
SECOW 2W X IT 93K-452-1	0.45	-0.56	-0.55	-1.31	1.56	-0.62	2.42	-0.34	-8.83
SECOW 4W X IT 93K-452-1	-0.55	-2.76***	-1.15	0.09	-2.32	-2.78***	-3.39	-7.55***	11.09
SECOW 3B X IT 93K-452-1	-0.89	0.27	-0.34	0.01	-4.37**	0.02	-6.48**	-2.56	-35.57***
SECOW 5T X IT 93K-452-1	0.65	-3.36***	2.45*	1.29	1.03	-3.46***	1.63	-10.14***	17.30*
SECOW 1T X IT 93K-452-1	0.45	-1.96**	0.45	-0.31	-5.45***	-2.87***	-8.09***	-6.40***	24.94**
SECOW 2W X IT 98K-205-8	0.45	-0.56	-0.55	-1.31	1.56	-0.62	2.42	-0.34	-8.83
SECOW 4W X IT 98K-205-8	0.25	1.44*	2.65*	1.29	-0.08	2.38**	-0.03	3.90**	-23.94**
SECOW 3B X IT 98K-205-8	-1.55*	3.04***	1.25	4.69***	1.19	4.70***	1.87	9.52***	-11.01
SECOW 5T X IT 98K-205-8	1.05	1.64**	1.65	4.89***	2.13	2.23**	3.27	3.03*	-30.47***
SECOW 1T X IT 98K-205-8	0.05	2.84***	-1.75	2.69*	-2.38	2.28**	-3.48	2.98*	-28.54**
SECOW 4W X IT99K-573-1	-1.19	0.24	1.46	-1.31	-1.07	-0.07	-1.52	1.69	-12.36
SECOW 3B X IT99K-573-1	-2.15**	1.64**	-2.55*	1.11	-0.93	0.88	-1.32	3.26*	-27.64**
SECOW 5T X IT99K-573-1	0.85	-0.16	0.25	0.49	3.33**	0.89	3.47	2.24	3.83
SECOW 1T X IT99K-573-1	-0.15	1.04	-0.55	-3.51**	-1.98	0.17	-2.88	2.42	8.31
SECOW 2W X IT 89KD-288	0.45	-0.56	-0.55	-1.31	1.56	-0.62	2.42	-0.34	-8.83
SECOW 4W X IT 89KD-288	0.45	-0.56	-0.55	-1.31	1.56	-0.62	2.42	-0.34	-8.83
SECOW 3B X IT 89KD-288	-2.15**	1.64**	-2.55*	1.11	-0.93	0.88	-1.32	3.26*	-27.64**
SECOW 5T X IT 89KD-288	3.05***	-2.76***	1.45	-1.51	4.05**	-2.13**	6.16***	-3.94**	-9.98
SECOW 1T X IT 89KD-288	0.45	-0.56	-0.55	-1.31	1.56	-0.62	2.42	-0.34	-8.83
Standard Error of the Mean	0.74	0.6	1.13	1.33	1.27	0.79	1.93	1.38	8.6

*, **, *** significant at P ≤ 0.05, 0.01 0.001 respectively, NPP, number of pods per plant; NSP, number of seeds per pod; 100SWT, 100 seed weight; DLS, delayed leaf senescence; NS, no stress; DS, drought stress.

sensitive cultivars.

Heritability and combining ability

General combining ability (gca) refers to the average performance of a parent in hybrid combinations and specific combining ability (sca) is the performance of a parent relatively better or worse than expected on the basis of the average performance of the other parents involved (Griffings, 1956). The significant mean squares of GCA_{male} and specific combining ability (SCA) for number of pods per plant, 100 seed weight and seed yield, indicated that both additive and non-additive genetic factors were important in the genetic control of these traits. However, the additive gene effects were relatively more important than the non-additive for the traits number of seeds per pod and delayed leaf senescence as the estimates of Baker's ratio and narrow sense coefficient of genetic determination were high. This implies that in this set of crosses, there would be a fairly high predictability of progeny performance from the parents' GCA effects for these traits. Besides, since additive genetic variance is the variance of breeding values and the main determinant of response to selection (Falconer, 1996), these traits will be easy to improve by simple selection methods such as mass selection and pedigree method. Similar findings were reported by Chiulele (2010). The non-additive gene action due to dominance and/or

epistasis, as shown by significant SCA mean squares contributed to the total genetic variation observed in number of pods per plant, 100 seed weight and grain yield. Gupta et al. (1993) reported that the presence of non-additive genetic effects lowers progress expected from early generation selection. The Baker's ratio was moderate for grain yield and 100 seed weight, low for number of pods per plant indicating the predominance of non-additive genetic control in the genetics of these traits. Variation observed among plants is due to the combined action of genetic and environmental factors (Baker, 1978). Heritability is a measure of the proportion of variance observed among plants that is due to genetic differences and is expressed in a broad or narrow sense way. The broad sense heritability is responsible for providing the proportion of genetic variance present in the phenotypic or total variance (Lobato et al., 2014). The broad sense coefficient of genetic determination was high for grain yield, 100 seed weight and number of pods per plant. Higher Broad sense coefficient of genetic determination estimates may be caused by greater additive genetic variance, lower environmental variance or minor inter-actions between genotype and environment (Acquaah, 2007). Similar results on high broad sense heritability estimates for grain yield. 100 seed weight and number of pods per plant were reported by Lobato et al. (2014). Though, broad-sense coefficient of genetic determination estimates were very high (> 0.5) for grain yield, number of pods per plant and 100 seed weight traits, narrow-sense coefficient of determination values were moderate to low (< 40%). Since narrow-sense coefficient of genetic determination estimates shows the proportion of a trait that is transmitted from parents to their progenies, the low narrow sense coefficient of genetic determination estimates for these traits signifies the presence of dominance effect (Abney et al., 2001) and suggests a low gene transmission to the progenies. Hence, a lower response to selection is expected in early generations as opposed to later advanced generations. Improvement for these traits therefore requires a recurrent selection procedure that allows for favorable gene recombination in later generations before a final selection is made. Similar results on moderate to low narrow sense heritability estimates for grain yield, 100 seed weight and number of pods per plant were reported by Alidu et al. (2013). However, these results contradicted with Chiulele (2010) findings, possibly due to the differences in the population studied, multilocationality of the study as Chiulele's study was conducted across locations while this study was done in one location.

Combining ability effects

The GCA estimates provide information on the concentration of predominantly additive gene effects and are useful in identifying parents to be used in breeding programs (Cruz et al., 2004). Dabholkar (1992) reported that parents with significant GCA effects in the desired direction for a character of interest are the best for hybridization. Female parent SECOW 5T showed positive significant GCA effects for seed yield and its components, negative significant GCA effects for delayed leaf senescence in a desirable direction under water stress suggesting that this cultivar would contribute to increasing seed yield, number of pods per plant, number of seeds per pod and 100 seed weight under moisture stress conditions. As such SECOW 5T can be used in a breeding program as a source of drought tolerance. Similarly, male parents IT93K-452-1 and IT 98K-205-8 showed desirable significant positive GCA effects for seed yield and its components and negative significant GCA effects for delayed leaf senescence. These male parents can be utilized in breeding for drought tolerance as good parents. Crossing between two good general combiners governed by additive x additive gene actions may elicit transgressive segregants in the advanced generations for the traits, thereby producing hybrids with good specific combining ability (Daniel et al., 2006).

Cruz et al. (2004) reported that selection for favorable estimates of SCA should prioritize cross combinations that involve at least one parent which has shown favorable effect of GCA. The F2 crosses SECOW 5T X IT 98K -205-8, SECOW IT X IT 98K-205-8 and SECOW 3B X IT 98K-205-8 showed desirable positive significant SCA effects for seed yield and its components, implying that these crosses performed higher than what was predicted based on their parents' GCA effects. The average seed yield of SECOW 3B x IT98K-205-8, SECOW 5T x IT98K-205-8 and SECOW 4W x IT98K-205-8 under moisture stress conditions was above the mean seed yield of all the crosses. The dominance of these crosses may be due to complementary and duplicate gene actions (Falconer, 1989). As such these crosses are expected to produce desirable segregants and could be exploited in cowpea varietal improvement programs. In effect, a large and positive SCA effects for a trait suggests the possibility for transgressive segregation for the trait in later generation of selfing (Ojo, 2003).

Conclusion

This study elucidated the genetic control of drought adaptation traits in selected cowpea parental genotypes. Both additive and non-additive genetic effects were responsible for the inheritance of drought adaptation traits. However, non-additive genetic effects were more important than additive genetic effects implying that the performance of progenies was better in specific crossing combinations and could not be predicted for a wide range of crosses. Therefore, improvement of drought adaptation

traits through selection of crosses with high positive SCA effects and advancing them to later generation would be effective. The good combiners for seed yield, number of pods per plant, number of seeds per pod, 100 seed weight and delayed leaf senescence were SECOW 5T, IT 93K-452-1 and IT 98K-205-8. These parents are, therefore, recommended as sources of drought tolerance for breeding programs. The F_2 families of the crosses: SECOW 3B x IT98K-205-8, SECOW 5T x IT98K-205-8, SECOW 4W x IT98K-205-8 and SECOW 1T x IT98K-205-8 were promising combinations that showed desirable positive significant SCA effects for seed yield, 100 seed weight and number of pods per plant. These crosses should be advanced for selection in later generations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Induction of flowering in cassava through grafting

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Flowering in cassava is related to branching. Erect plant architecture is usually preferred by farmers but results in late and scarce flowering, which slows down breeding and genetic studies. The objective of this study was to induce earlier and more abundant flowering, which have become key research needs for cassava. Six non- or late-flowering genotypes were selected for grafting on a profuse, early flowering understock. Grafted stems did not branch and flower while attached to the understock. Four cuttings from each grafted stem were taken and planted the following season. Paired-row cuttings from non-grafted stems of the same genotypes were planted as checks. Three phenotypic responses to grafting were found. One genotype failed to branch and flower, independently of the origin of the cuttings. Four genotypes branched but did not produce flowers. However, plants from grafted cuttings tended to branch earlier, particularly after the second branching event. Finally, in one genotype, grafting induced not only earlier branching but also earlier and more abundant production of flowers, fruits and seeds than their counterparts of plants from non-grafted stems. This is the first report of grafting effects on the induction of earlier flowering in cassava. Results indicated a delayed effect of grafting which was genotype-dependent based on materials used in this study. The contrasting responses to grafting may be useful for understanding the effect of plant growth regulators and photoperiod manipulations of ongoing research.

Key words: Accelerated breeding, branching, genetic gains, genomic selection, inbreeding.

INTRODUCTION

Commercial multiplication of cassava is achieved through stem cuttings. Sexual reproduction, a key requirement for cassava breeding, is common and relatively easy to achieve (Kawano, 1980). Cassava is a diclinous and monoecious species: Both female (pistillate) and male (staminate) flowers are produced in inflorescences (racemes or panicles) within the same plant. Pistillate flowers occupy the lower portion of the raceme or panicle and open 10 to 14 days before the male flowers which are located toward the apex on the same inflorescence. Inflorescences always develop at the apex of the stem. Sprouting of the buds below the inflorescence allows further growth of the plant. Therefore, every flowering event results in branching. Some genotypes flower frequently (3 to 5 times during a growth cycle) and others flower little or late.

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Erect, non-branching types, however, are often preferred by farmers because they facilitate cultural practices, enhance the production of stems (the vegetative planting material), and transport and storage of non-branched stems is easier. The long stems of non-branching types tend to retain their sprouting capacity for longer storage periods, thus it has become an important adaptive trait (Ceballos et al., 2011). Molecular markers for height of first branching have been identified (Boonchanawiwat et al., 2011).

Synchronization of flowering for planned crosses can be a challenge because some clones flower relatively early at 4 or 5 months after planting (MAP) whereas others flower only after 10 MAP. The scarcity of flowers in erect, non-branching types only complicates matters further. It is not surprising that efforts to accelerate flowering in cassava began many years ago. Accelerating flowering in cassava would facilitate the routine operations in crossing nurseries, reducing the costs of operation and speeding up the production of segregating progenies. Moreover, the need for a protocol to accelerate flowering in cassava has become more urgent in recent years. The advantages to introduce inbreeding in cassava genetic enhancement have been demonstrated (Ceballos et al., 2015, 2016). Accelerated flowering would facilitate the development of inbred progenitors through successive self-pollinations. Induction of flowering in cassava would also allow taking full advantage of the benefits that genomic selection could offer to the crop. There is an ongoing research to validate the potential of genomic selection in cassava (Next Generation Cassava Breeding project, www.nextgencassava.org). It was recognized that the induction of flowering was a key requirement for genomic selection because it would allow achieving a more balanced number of progenies from each progenitor and shorten the length of each recurrent selection cycle.

Flowering in plants is a complex process involving environmental, developmental and genetic factors interactions (Bäurle and Dean, 2006; Lee and Lee, 2010; Ha, 2014; Sung and Amasino, 2004).Elegant studies in the 1930s demonstrated that a mobile signal was involved in spinach flowering (Knott, 1934). Further studies in other crop species confirmed these initial finding and led to the coining of the term "*florigen*" for this photoperiod stimulus in the leaves, which is then transmitted to the apical meristem (Chailakhyan, 1936; Zeevaart, 2008). Recent studies in the model plant *Arabidopsis thaliana* have provided important insights of the key genetic factors related to florigen. The *Flowering Locus T* (*FT*) strongly influences flowering (Amasino, 2010; Putterill et al., 2004; Turck et al., 2008; Yeoh et al., 2011; Kobayashi et al., 1999). The *FT* protein is a mobile signal produced in the leaves and transported via phloem to the apical meristem where it interacts with other transcription factors to initiate floral development (Abe et al., 2005; Amasino, 2010; Hempel et al., 2000; Wigge et al. 2005; Zeevaart, 2008). The induction of *FT* expression in leaves and its movement to the apex where it triggers flowering appears to be universally conserved (Wigge, 2011; Yeoh et al., 2011).

Environmental conditions such as low (Kim et al., 2009) or high temperature (McClung et al., 2016; Warner and Erwin, 2006) or photoperiod signals (Searle and Coupland, 2004) regulate the expression of *FT*, thus influencing flowering responses (Jung and Müller, 2009; Ha, 2014). In fact, the photoperiodic induction of flowering was discovered more than a century ago (Tournois, 1914). Developmental factors also influence flowering in plants. During the juvenile stage plants cannot react to the stimuli that induce flowering in mature plants (Ha, 2014; Pillitteri et al., 2004). As the plant ages, however, it becomes sensitive to external factors inducing flowering, thus reaching the reproductive stage. Several approaches have been successfully used to modify flowering patterns in plants (Wilkie et al., 2008). Modification of the environmental conditions (temperature and photoperiod) has been exploited for many years (Garner and Allard, 1920). The exogenous application of plant growth regulators successfully induce flowering not only in angiosperm species (Aliyu et al., 2011; Liverman and Lang, 1956; Henny and Chen, 2011) but also in gymnosperms (Luukkanen and Johansson, 1980). Grafting techniques have also been used to take advantage of the mobility of the signal for flowering (Notaguchi et al., 2009). Many decades before the discovery of the *FT* locus, grafting was exploited to hasten flowering in sweet potato (Kobayashi and Nakanishi, 1982; Zobel and Hanna, 1953), sugar beet (Curtis and Hornsey, 1964), or the Crassulaceae family (Zeevaart, 1978). Genetic transformation to increase the level of *FT* has also been successful (Kardailsky et al., 1999; Kobayashi et al., 1999).

Early attempts to accelerate flowering or increase number of flowers and seed set in cassava have been attempted through the exogenous applications of growth regulators such as IAA, NAA, and ascorbic acid (Indira et al., 1977) as well as longer photoperiods and cooler temperatures (de Bruijn, 1977; Keating, 1982). Induction of flowering for plants growing *in vitro* through addition to the growth media of gibberellins and cytokinin in the presence of auxin growth regulators has been reported (Tang et al., 1983). Finally, the development transgenic cassava in which the *FT* gene has been over expressed appears to hasten flower induction (Adeyemo et al., 2008).

Grafting has been reported in cassava as a means of joining above-ground germplasm with high photosynthetic potential with below-ground germplasm with high storage root production (Ahit et al., 1981; Pellet and El-Sharkawy, 1994). However, to our knowledge, there has not been any published report to induce flowering in cassava through the grafting technique. This article reports research conducted over the last four years on the

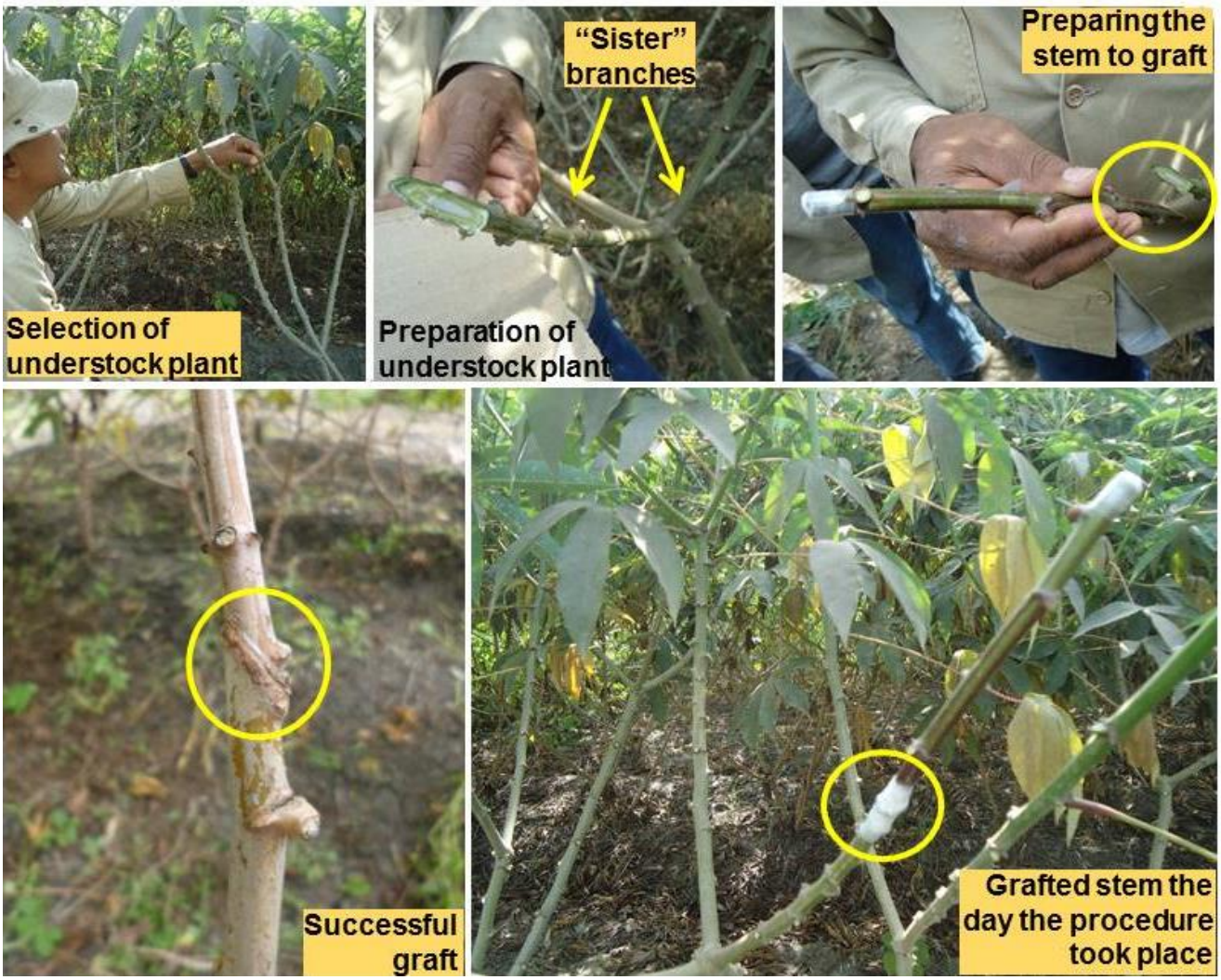

Figure 1. Illustration of the procedure used to graft a piece of stem from a non-flowering genotype onto a profuse, early-branching understock. One of three branches was used for the graft and the two remaining "sister" branches were left untouched.

grafting of branches from non-flowering cassava genotypes on understocks from a profuse, early flowering genotype.

MATERIALS AND METHODS

Location

All data was collected at CIAT´s Experimental Station, in Palmira, Valle del Cauca, Colombia. This site is located less than 4° north of the Equator. The duration of the photoperiod is therefore uniform throughout the year.

Germplasm

Six cassava genotypes were selected because of their late or negligible flowering habit (erect plant architecture with late or no branching): SM3348-29; SM3402-42; SM3409-42; SM3409-43; GM3500-9 and GM3500-2. Stems of these non-flowering types were grafted on an early, profuse-flowering clone (HMC1) understock. In breeding work cassava scientists use flowering and branching as synonymous events although they are not. In this paper a distinction will clearly be made, when necessary, to describe the occurrence of these events.

Grafting protocol

Plants from the understock (HMC1) genotype had already flowered when grafts were made, about 4 to 5 months after planting (MAP). Typically, 3 branches emerge at each branching event in HMC-1. One of the branches in the HMC1 understock was cut diagonally to receive the grafted stem from the non-flowering genotypes, which was similarly cut so the pieces matched closely in diameter and angle (Figure 1). The remaining two "*sister"* branches of the understock were left untouched. Stems of non-flowering genotypes of about 1 cm in diameter were used for the grafting. The diameter of the stem to graft and of the selected branch of the understock was the same and developmental stage of understock and scion

Table 1. Summary of the six non-flowering genotypes from which grafts were obtained.

Genotype	Grafted origin			Non-grafted origin	
	Number of grafts	Cuttings planted	Sprouted cuttings	Cuttings planted	Sprouted cuttings
GM3500-2[a]	8	32	32	32	32
GM3500-9[a]	6	24	23	24	24
SM3348-29	6	24	24	24	24
SM3402-42	8	32	32	32	32
SM3409-42 [b]	3	12	12	12	12
SM3409-43[b]	4	16	16	16	14
Total	**35**	**140**	**139**	**140**	**138**

[a,b]Genotypes genetically related. GM3500-2 and -9 are member of the same full-sib family. Therefore they share the same female and male progenitors. SM3409-42 and -43 are member of the same full half-sib family. Therefore they share the same female progenitor only. The number of grafts obtained, number of planted and sprouted cuttings from each genotype is also shown. For each genotype the same number of cuttings from non-grafted stems (used as control) was planted. Information of their sprouting is provided in the column on the right.

stems were such that their vascular tissues aligned closely with each other. Remaining branches in the understock were not pruned. Grafted stems were immediately wrapped tightly with parafilm (Figure 1) to accelerate healing and provide additional physical support to keep the graft connected with the understock. Grafted stems can easily be lost during the first few weeks after the procedure due to their delicate mechanical support. Walking around the nursery was done carefully to avoid damaging them. Grafted stems were allowed to grow for several months and data taken on flowering (if any).

Experimental design

At the end of the growing cycle (about 11 to 12 months after planting the understock) a total of 35 grafted stems from the six non-flowering genotypes were available (Table 1). From each of these grafted stems four cuttings (20 to 25 cm long) were taken. Their relative position in the proximal to distal end of the branch was recorded. Similarly, four cuttings from non-grafted stems of the same non-branching genotypes were also collected and identified from bottom up. These cuttings were planted on July 15, 2015 in paired rows. One row was planted with cuttings from grafted stems and the other with cuttings from non-grafted stems of the same genotype. The first cutting planted in the row was the one positioned in the most proximal (bottom) section of the graft (stem). Similarly, the fourth cutting in the row came from the most distal (top) section of the graft (stem). Similar pattern was used for the rows planted with non-grafted stems. Cuttings were chosen to have similar diameter. Since four cuttings were obtained per graft a total of 35 × 4 = 140 plants were expected from grafted cuttings which were planted in the same row 1 m apart from each other (Table 1). In the neighboring row cuttings from non-grafted stems of the same genotype were planted following the same criterion (Figure 2). An empty space was left in the row to separate plants from different grafts.

Field management

Field management followed the standard procedures for cassava. A pre-emergence herbicide treatment was applied four days before planting. Manual weeding was made as necessary. Plots were uniformly fertilized following standard procedures. Insecticides were applied as necessary. Irrigation was provided via surface/gravity distribution also as required.

Data recorded

Plants were analyzed individually for the following traits: (a) Number of sprouted buds per cutting; (b) Number of main stems developed was recorded for each cutting (the field was screened frequently until the first and subsequent branching events could be noticed); (c) Number of branching events; (d) Number of flowers at anthesis; and (e) Number of fruits and seeds.

Weekly assessment of branching and flower production began in October 16 (when branching was observed for the first time in a few plants) and finished on March 30. No further data on flowering and branching was taken thereafter: plants had grown too much and data gathering was difficult, but more importantly, because this research focused on the induction of earlier flowering and late season information was irrelevant for the research. At the end of the growing cycle, however, attention was paid to the developing fruits. As fruits started to dry, they were covered with mesh bags to collect seeds when dehiscence occurred. Plants were kept in the field until July 1st. Immature fruits were harvested at harvesting time and opened to count the number of seeds developing inside.

RESULTS AND DISCUSSION

Flowering of grafted stems

There was considerable variation in the number of grafts surviving at the end of the growing cycle of the understock 11 to 12 MAP (Table 1). Eight grafts were available from SM3402-42 and GM3500-2. Six grafts from SM3348-29 and GM3500-9 remained attached to HMC1 12 MAP (or about 7-8 months after grafting). Finally, three and four grafts were available from SM3409-43 and SM3409-43, respectively.

None of the 35 grafted stems flowered while growing on top of the understock. These grafts grew considerably more slowly than the '*sister*' untouched branches of the understock plant. The delayed growth of the grafts appeared to be the result of the stress due to the grafting procedure. Alternatively, the vascular tissue may not have successfully formed a graft union merging the xylem and phloem of the respective partners. While the leaves

Table 2. Summary of the number of flowers counted in each of the 24 plants from genotype SM3348-29 derived from grafted or non-grafted cuttings.

Graft (plant)	Cuttings from grafts				Cuttings from stems		
	Day after planting			Number	Day after planting		Number
	183	230	260	of fruits	190	260	of fruits
1(1)	5	10	14	10	0	0	0
1(2)	2	0	0	0	2	0	0
1(3)	0	0	2	0	0	0	0
1(4)	5	0	0	0	0	0	0
2(1)	3	6	7	0	0	0	0
2(2)	2	34	35	16	0	0	0
2(3)	1	10	17	4	0	3	0
2(4)	0	0	7	3	6	18	10
3(1)	3	0	9	4	0	11	14
3(2)	2	0		0	3	4	2
3(3)	4	0	7	4	0	8	0
3(4)	3	18	29	20	0	0	0
4(1)	5	0	11	0	0	0	0
4(2)	4	0	0	0	0	0	0
4(3)	4	2	11	3	0	0	0
4(4)	4	35	23	23	2	3	8
5(1)	4	40	14	26	3	2	0
5(2)	4	20	23	11	0	0	0
5(3)	4	53	3	56	3	0	0
5(4)	5	1	32	94	0	24	13
6(1)	7	81	17	124	0	7	6
6(2)	5	20	5	13	0	0	0
6(3)	7	58	7	85	0	0	0
6(4)	8	71	1	67	0	5	2
Total	**91**	**459**	**274**	**563**	**19**	**85**	**55**

The number of flowers was counted at each of the respective flowering peaks (three and two peaks, for plants from grafts and stems, respectively).

of the scions did not wilt or show other signs of water insufficiency, and leaves appeared to be photosynthetically competent, it is possible the xylem and phloem limited flux to low rates. Based on this observation we suggest in future trials that the '*sister*' (non-grafted) branches of the understock should be cut at the time the grafts are made. This may give the grafted stems an improved chance to grow competitively in relation to the remaining branches.

Flowering of plants from grafted vs. non-grafted cuttings

Sprouting occurred in 98% of the cuttings obtained from grafted stems that had been obtained the previous season (Table 1). Only three cuttings from grafted stems (out of 140) failed to sprout. This is, in fact an excellent sprouting ratio. The stems that failed to sprout were all from genotype SM3402-42. One of the cuttings that failed to sprout was the fourth (most distal) in graft # 2. The remaining two failures in sprouting came from the third and fourth most distal cuttings obtained from graft # 5. So it seems that younger stem tissue tended to be more susceptible to a sprouting failure. In addition, two cuttings failed to sprout from the non-grafted material. They also came from a single genotype (SM3409-43). In one case it was the third plant (e.g. a cutting coming from almost the top of the stem) from plant # 1. The second cutting that failed to sprout was the first one (e.g. bottom of the stem) from plant # 2. The sprouting percentages were, therefore, very similar for cuttings coming from grafted branches or from ordinary stems (97.86 and 98.57%, respectively). Plant growth was normal without unusual stress from pests or diseases.

Genotype SM3409-43 did not branch or produce any flower in plants derived either from grafts or non-grafted stems. Plants from the remaining genotypes all branched but did not produce flowers, except for genotype SM3348-29.

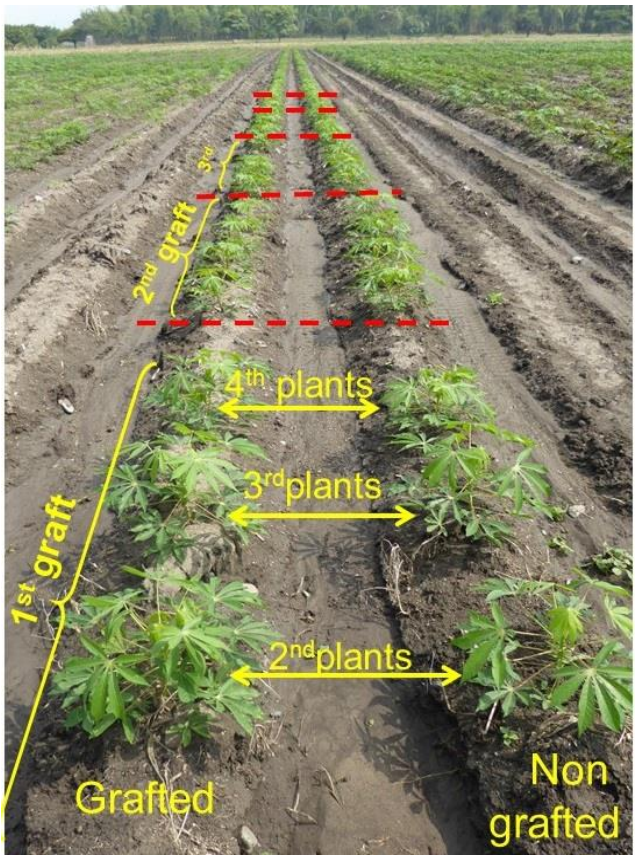

Figure 2. Photograph of plants from grafted (left) and non-grafted (right) cuttings of the same genotype. Four cuttings per graft were planted. Similarly, four cuttings from non-grafted stems of the same genotype were planted in the neighboring row.

Figure 3. Photographs taken on January 4 (174 days after planting). Top photographs illustrate branching without flowering (or perhaps remnants of a rudimentary one). Bottom photographs were taken in plants from grafted cuttings of genotype SM3348-29, with inflorescences at different stages of development.

The branches observed were similar to those normally associated with fork-type branching and flowering (Figure 3).

Figure 4 presents the performance of the four genotypes (SM3409-42; SM3402-42; GM3500-2 and GM3500-9) that branched but did not produce flowers. Frequency of first branching was similar in plants from grafted and non-grafted stems in these genotypes. Second branching tended to be earlier and more common (e.g. present at higher percentages) in plants from graft cuttings than in those from non-grafted stems in every genotype, except GM3500-9. In the case of SM3402-42 plants coming from graft cuttings, were the only ones showing a third branching event, although at a low frequency. However, no flowering was observed in any of these plants. These results show that branching does not necessarily result to (detectable) flowering (Figure 4).

It was already mentioned that SM3348-29 showed a unique performance. Plants derived from grafts branched up to four times (Figure 5). Plants from non-grafted stems had only three branching events during the period of observation. At every branching event, plants from grafts were earlier than those from the non-grafted counterpart. Moreover, the tendency accentuated with each branching event (double-end arrows in Figure 5).

More importantly only SM3348-29 flowered and produced fruits and seeds, although considerably more abundantly in plants from grafts. The total number of flowers counted in 24 plants each of grafted and non-grafted cuttings at different times is presented in Figure 6. It is clear that plants from grafted cuttings flowered earlier and more abundantly than those from non-grafted stems. For example, 174 days after planting (January 5) a total of 91 flowers were counted on the 24 plants derived from grafted cuttings, whereas only 2 had developed in plants from stems. In general, personnel doing pollinations in cassava do not give priority to flowers related to the first branching event as they are often sterile and have low fruit and seed set.

Differences in the number of flowers related to the second branching event are probably more relevant to breeding programs. On February 18 (219 days after planting) plants from grafted cuttings had clearly initiated a second flowering event (231 flowers), which reached a peak few days later (459 flowers). Plants from ordinary stems flowered considerably later and not so profusely. They produced a maximum of only 85 flowers and 260 days after planting (March 30). Number of flowers presented in Figure 6 suggests a tri-modal distribution in plants from grafted cuttings which can be linked to the

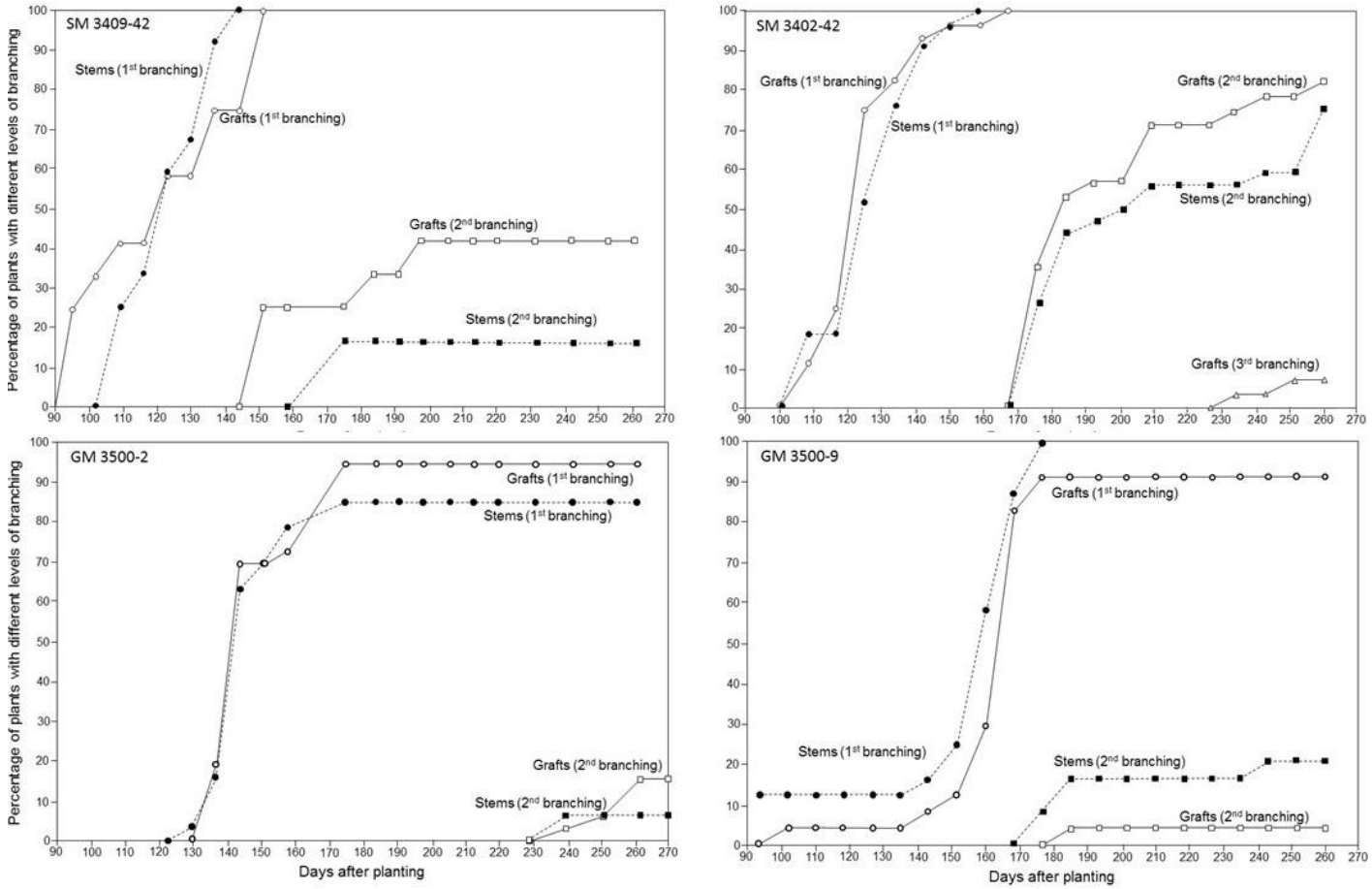

Figure 4. Frequency of first (circles), second (squares) and third (triangles) branching in plants from cuttings obtained after grafts (open circles or squares) or from non-grafted ordinary stems (filled circles or squares) in four genotypes that branched but did not produce flowers. Data was taken approximately every 7 to 8 days.

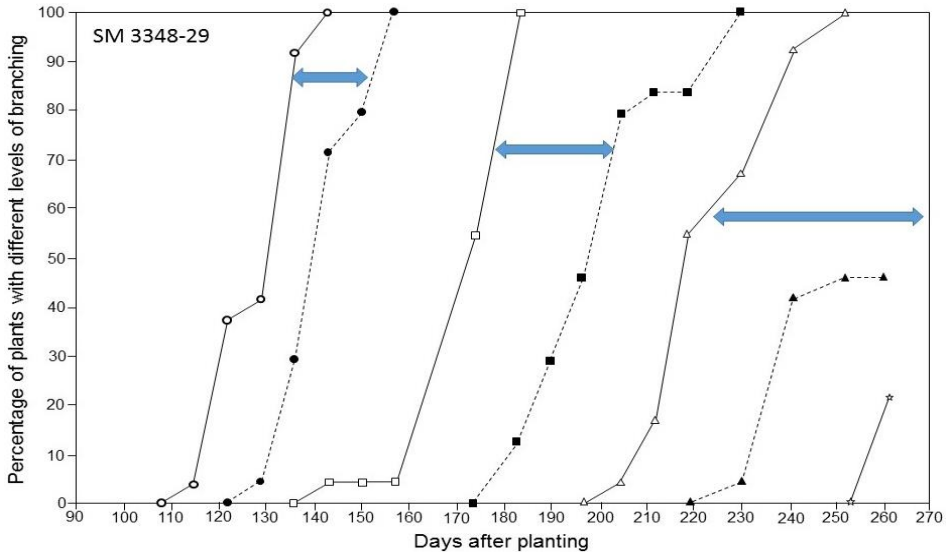

Figure 5. Frequency of first, second, third and fourth branching in SM3348-29 plants from cuttings obtained after grafts (open circles, squares, triangles or stars) or from non-grafted stems (filled circles, squares or triangles) in the same genotype. Data was taken approximately every 7 to 8 days.

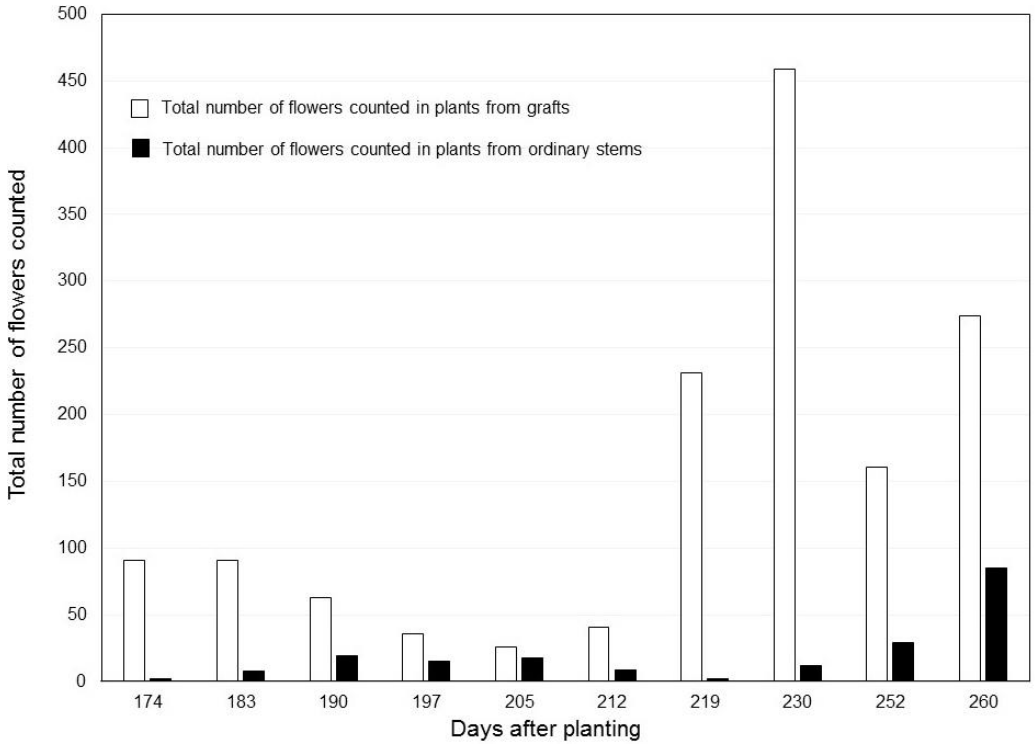

Figure 6. Total number of flowers counted in 24 plants from grafted cuttings (open columns) or 24 plants from cuttings collected from ordinary non-grafted stems (filled columns) of the same genotype (SM3348-29). The number of flowers counted along time fluctuates as it is related to the consecutive branching events.

first, second and third branching events (peaks at 174-183; 230 and 260 days after planting). In the first flowering peak 22 out of 24 plants had flowered. In the second peak, 15 plants were bearing flowers. In the last flowering peak, 20 of the 24 plants had flowers. There was some variation in flowering of plants derived from the different grafts (Table 2). All four plants from grafts # 5 and # 6 were bearing flowers at each of the three flowering peaks. Three and two plants from grafts # 2 and # 4 also had flowers in each flowering peak. The four plants from graft # 3 flowered 183 DAP, but only one was bearing flowers 230 DAP. In the last flowering peak (260 DAP) three of the four plants from graft # 3 had flowers. The poorest result was observed for plants from the first graft: three, one and two plants (out of four) had flowers in each of the three successive flowering peaks (183, 230 and 260 DAP, respectively). In plants from non-grafted cuttings, two peaks could be observed around 190 and 260 DAP (Figure 6). In the first peak, which was shallow, only six of the 24 plants had flowered. In the second peak, 10 plants were bearing flowers (Table 2).

Differences in the timing and number of flowers between plants from grafts or non-grafted ordinary stems eventually lead to a significant difference in the number of fruits formed as illustrated in Figure 7. By March 30 a total of 563 fruits were developing in plants from grafted cuttings, whereas only 55 were counted in the counterpart from stems (Table 2). Fruits were counted in plants from every graft, but responses were not uniform. The largest number of fruits was counted in plants from grafts # 5 and 6 (187 and 289 fruits). This agree with the higher and more consistent flowering of plants from these two grafts (Table 2). A total of 23, 28 and 26 fruits were counted in plants from grafts # 2, #3 and #4, respectively. Only 10 fruits were produced in plants from graft # 1. Fruits were obtained in 17 out of 24 plants derived from grafting. Only 6 of the 24 plants from non-grafted cuttings were bearing fruits that date. There is no need for statistical analysis to demonstrate a differential performance. Moreover, a total of 500 seeds were harvested in plants from grafted cuttings against none from non-grafted ordinary stems.

There were three different distinctive outcomes regarding the effect of prior grafting on branching and flowering of the six genotypes analyzed. SM3409-43 did not branch and failed to produce any flowers. Genotypes GM3500-2, GM3500-9, SM3402-42 and SM3409-42 went through at least two branching events but did not produce flowers (or they aborted before their presence could be registered). Finally, genotype SM3348-29 showed at least three branching events which were linked to flower production. Consequently, this is the only genotype

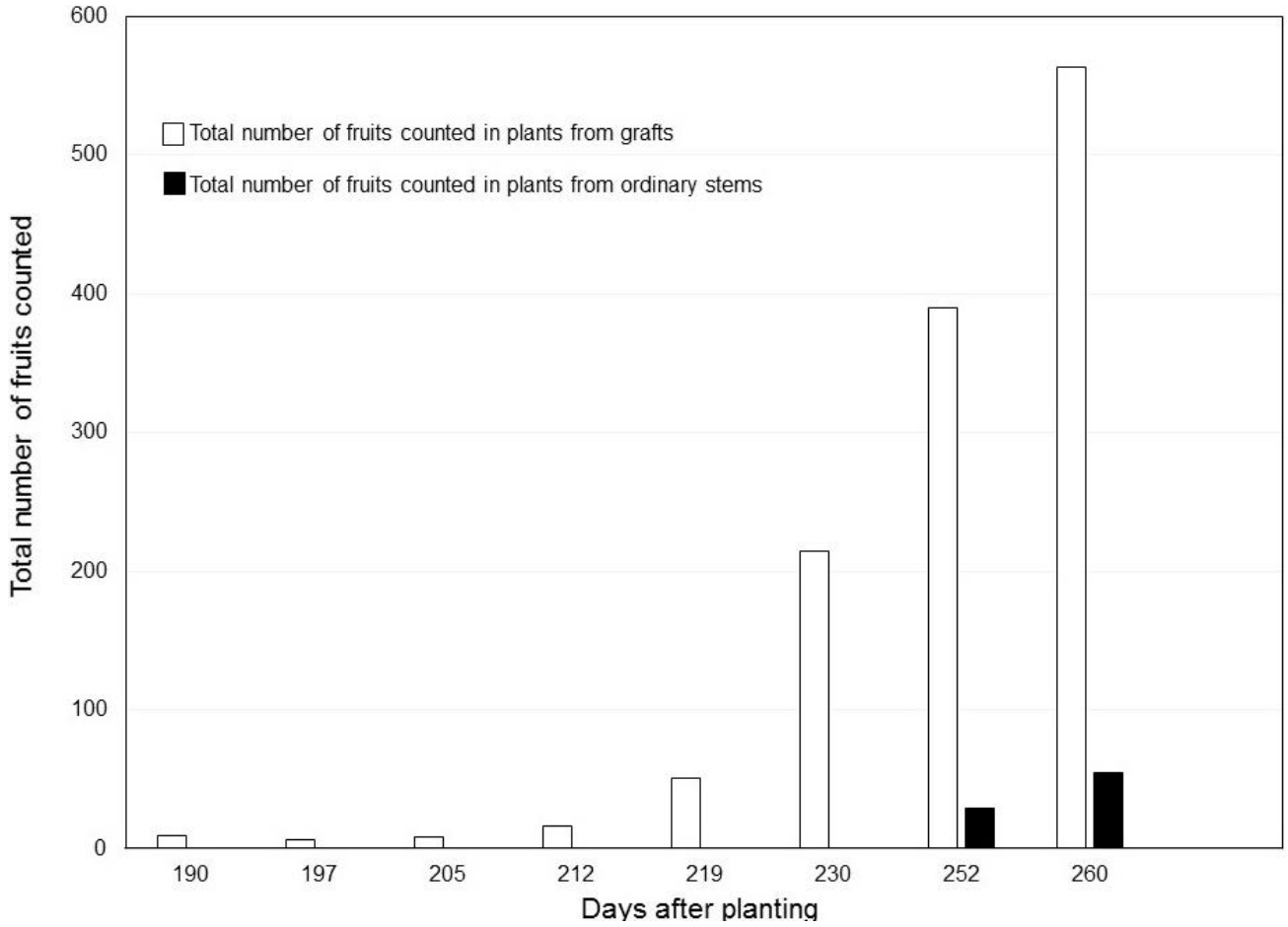

Figure 7. Total number of fruits counted in plants from 24 grafted cuttings (open columns) or 24 plants from ordinary non-grafted cuttings (filled columns) of the same genotype (SM3348-29).

that produced fruits and seeds. It is clear, therefore, that there are genetic differences for branching, flowering time and number of flowers among the six genotypes analyzed. It should be pointed out that genotypes used in this study were selected because of their scarcity of flower production. In most cassava genotypes, branching is, indeed, linked to flower production.

For each of these six genotypes there were plants derived from branches that had been grafted, or else, from cuttings obtained from ordinary (non-grafted) stems. Genotype SM3409-43 failed to branch or flower and will not be considered thereafter. Four genotypes (GM3500-2; GM3500-9; SM3402-42: and SM3409-43) produced branches without the expected production of flowers, regardless of the origin of the plants (grafted vs. ordinary stems). The comparison between these two contrasting origins was the main focus of this study. It can be concluded, therefore, that for these genotypes grafting did not induce detectable flowering. However, there was a trend for slightly earlier branching in plants from graft origin compared with those from non-grafted ordinary stems in most cases (Figure 4). So there may have been some stimulus for earlier flowering (e.g. the related branching) but eventually inflorescences failed to develop or else aborted before their presence could be detected.

In the remaining genotype (SM3348-29), prior grafting resulted in earlier branching and a considerable increase in the number of flowers, fruits and seeds (Figures 5 to 7). Moreover, branching was increasingly hastened from the first to the fourth branching events in plants derived from grafts compared with those from non-grafted ordinary stems (Figure 5). It seems that the effect of grafting was strengthened with each flowering event. These findings are very relevant for the purpose and needs of cassava breeding, as plants from the grafted cuttings flowered earlier and more abundantly than those from ordinary cuttings (Figure 6).This, in turn, had a clear impact on the number of fruits and seeds and collected at the end of the growing cycle (Figure 7). There was no evidence that the position (e.g. proximal or distal) of the four cuttings obtained from each graft had an effect on the number of flowers, fruits and seed (Table 2).

It is clear, therefore, that grafting in the cassava genotype SM3348-29 accelerated flowering and resulted

in a considerable increase in the number of seeds produced. This type of result agrees with those reported many years ago in sweet potato (Kobayashi and Nakanishi, 1982; Zobel and Hanna, 1953), sugar beet (Curtis and Hornsey, 1964), and other species (Zeevaart, 1978). However, it is also clear that the impact of grafting is genotype dependent as in the remaining genotypes, it did not induce detectable flowering (although in some cases there was a tendency for earlier branching). The availability of these different genotypes and the knowledge of their differential response may provide ideal research material for understanding why some genotypes branch without producing flowers, or else why these flowers abort before their presence can be detected. Perhaps with the application of plant growth regulators that foster fruit and seed set, flowers will be obtained in those genotypes that branched but failed to produce viable flowers.

Conclusion

This is the first reported study in which grafting was used to induce earlier flowering in cassava genotypes that do not flower or flower late in the season. Grafting did not have any result while growing on the understock. However, it showed a delayed effect that could only be observed in plants cloned from the grafted stems. Grafting had an effect of accelerating branching in most genotypes, particularly after the second branching events. Unfortunately, in most cases branching occurred without the parallel production of flowers. It is not clear if inflorescences failed to develop or if they did develop but aborted before their presence could be detected. In one case, however, grafting induced earlier flowering and more abundant production of fruits and seeds. Stem cuttings from the 24 plants derived from grafts or ordinary stems of genotype SM3348-29 will be taken from this experiment and planted to assess if the results of grafting have a residual effect on a second growing season.

The effects of grafting have a genotypic dependency which limits the potential for its generalized use in crossing nurseries in cassava breeding programs. However, this study has exposed three different types of genetic response to grafting (no branching, earlier branching without flower production and earlier branching with earlier and more abundant flower/seed production) which will be used for detailed studies on the use of plant growth regulators and photoperiod modulation.

Induction of flowering is fundamental for accelerating genetic gains in cassava. The impact of conventional breeding would be increased particularly if inbreeding could be incorporated into the process (Ceballos et al., 2015, 2016). The implementation of genomic selection would benefit by inducing early flowering, a fact that was recognized by the Next Generation Cassava Breeding project www.nextgencassava.org).

Genetic studies would also benefit from larger number of seeds from segregating progenies in a shorter period of time. It is acknowledged that the genotypic dependency of the effect of grafting limits the ultimate impact of this technology. However, this is a first step that could help in the development of more appealing approaches such as the use of plant growth regulators or photoperiod lengthening (alone or in combination with grafting) that so far have not yielded any result.

Conflicts of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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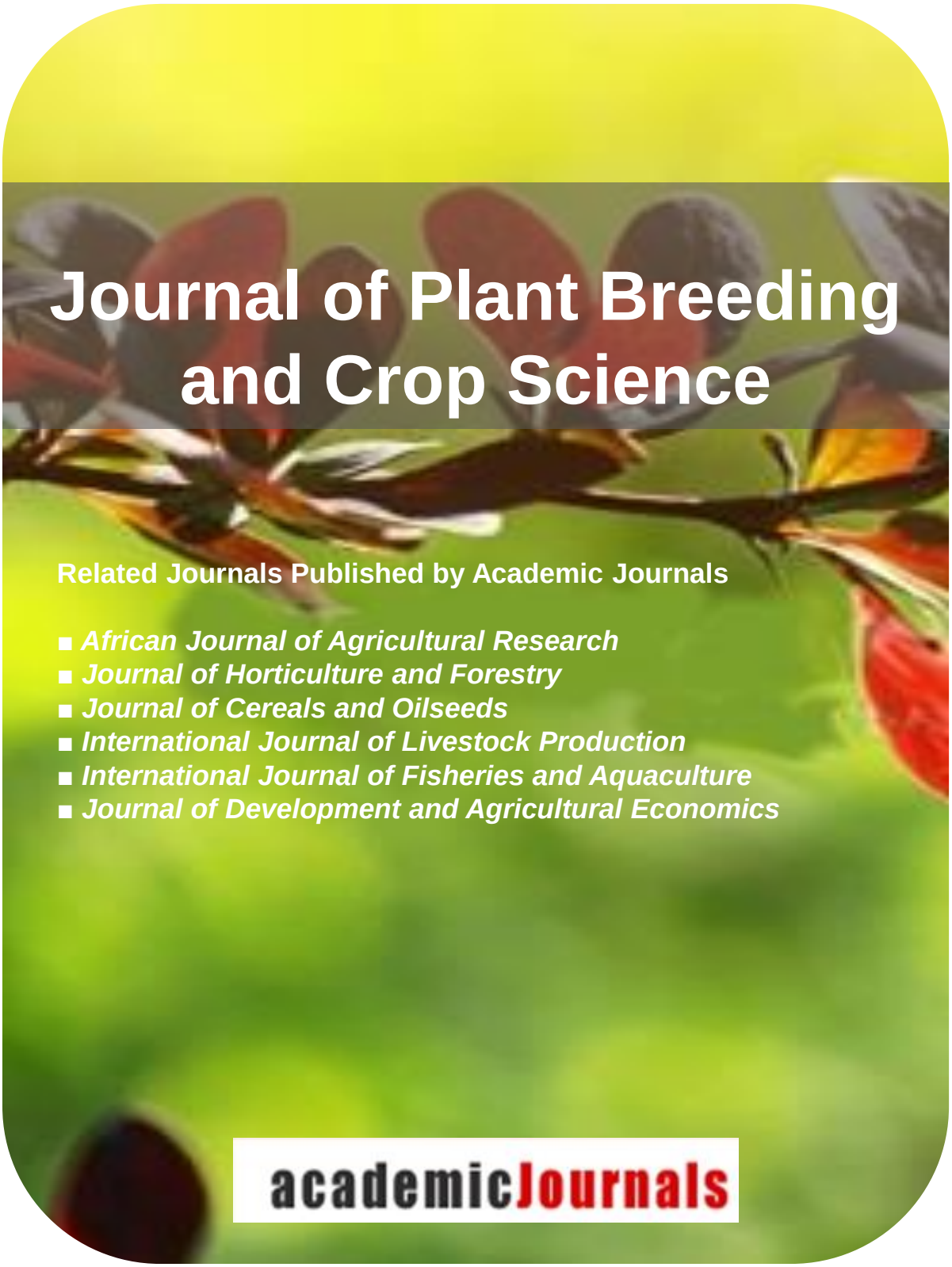
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