

*Full Length Research Paper*

## Induction of flowering in cassava through grafting

H. Ceballos<sup>1\*</sup>, J. J. Jaramillo<sup>2</sup>, S. Salazar<sup>1</sup>, L. M. Pineda<sup>1</sup>, F. Calle<sup>1</sup> and T. Setter<sup>3</sup>

<sup>1</sup>International Center for Tropical Agriculture (CIAT), Recta Cali-Palmira km 17, Valle del Cauca, Colombia.

<sup>2</sup>Universidad Nacional de Colombia - Palmira Campus, Valle del Cauca, Colombia.

<sup>3</sup>Soil and Crop Sciences, School of Integrative Plant Science, Cornell University. Ithaca, NY, USA.

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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.

\*Corresponding author. E-mail: [h.ceballos@cgiar.org](mailto:h.ceballos@cgiar.org).

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](http://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.

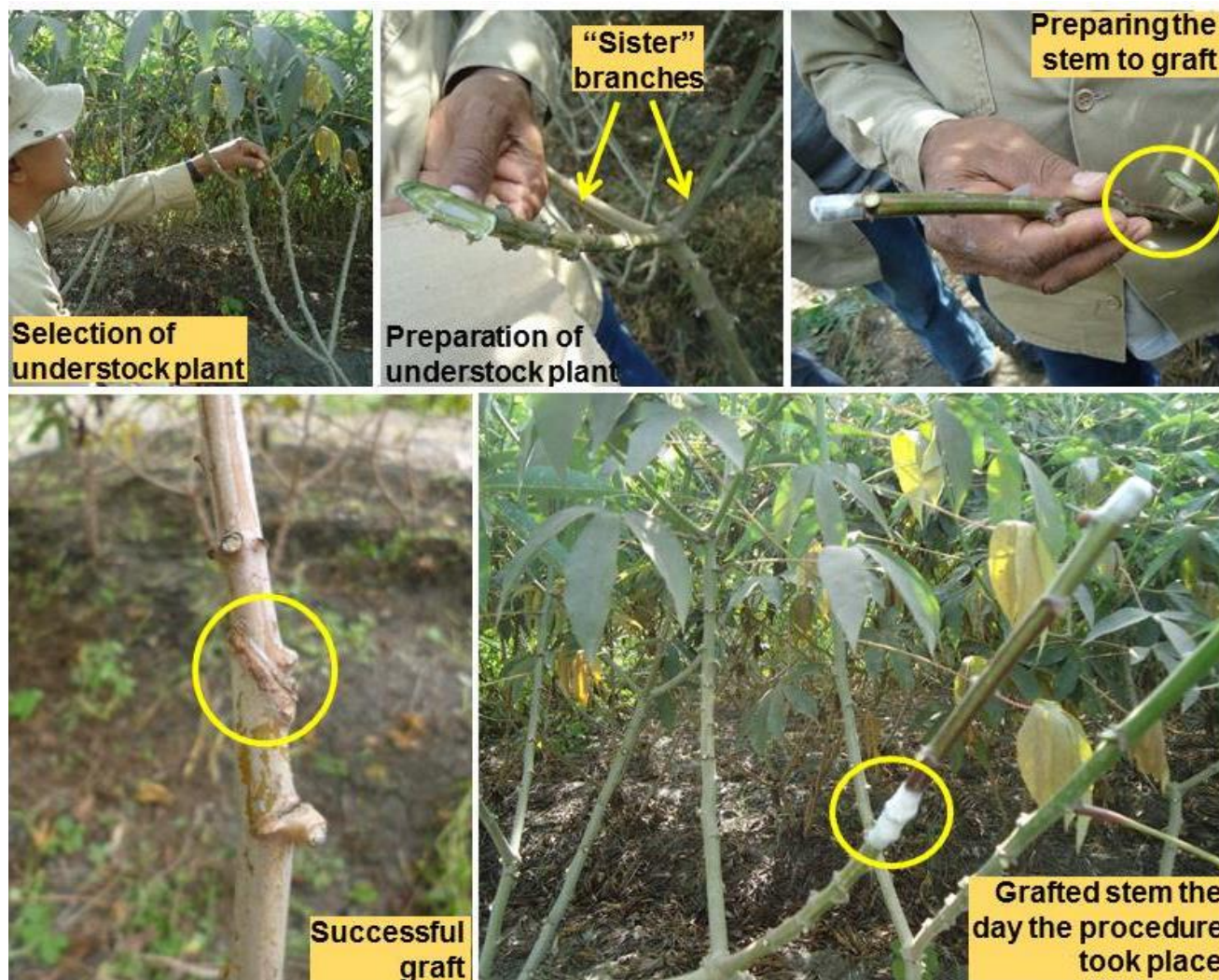
Flowering in plants is a complex process involving environmental, developmental and genetic factors interactions (Bäurle and Dean, 2006; Lee and Lee, 2010; Ha, 2014; Sung and Amasino, 2004). Elegant studies in the 1930s demonstrated that a mobile signal was involved in spinach flowering (Knott, 1934). 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 <sup>a</sup>	8	32	32	32	32
GM3500-9 <sup>a</sup>	6	24	23	24	24
SM3348-29	6	24	24	24	24
SM3402-42	8	32	32	32	32
SM3409-42 <sup>b</sup>	3	12	12	12	12
SM3409-43 <sup>b</sup>	4	16	16	16	14
<b>Total</b>	<b>35</b>	<b>140</b>	<b>139</b>	<b>140</b>	<b>138</b>

<sup>a,b</sup>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  $35 \times 4 = 140$  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 1<sup>st</sup>. 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 of fruits	Day after planting		Number of fruits
	183	230	260		190	260	
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
<b>Total</b>	<b>91</b>	<b>459</b>	<b>274</b>	<b>563</b>	<b>19</b>	<b>85</b>	<b>55</b>

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

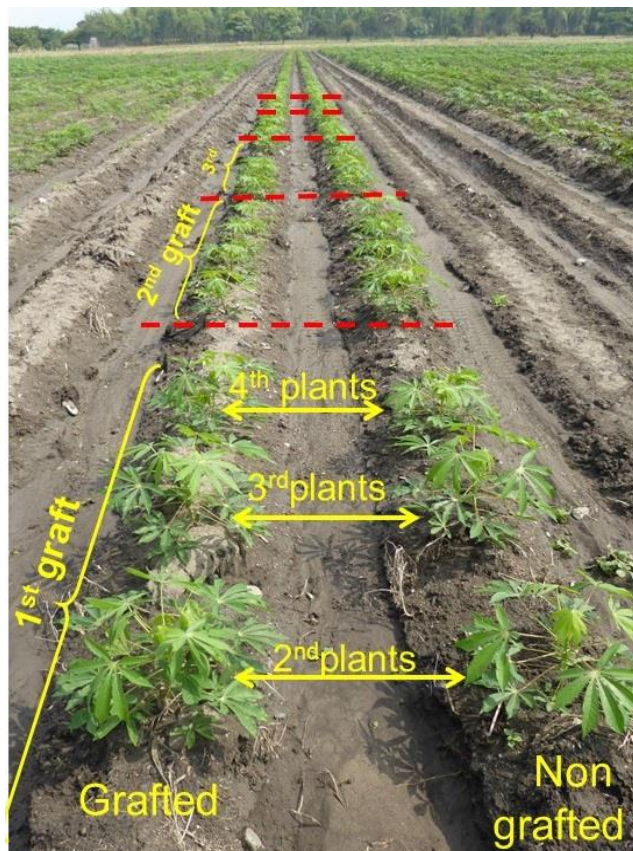
of the scions did not wilt or show other signs of water insufficiency, and leaves appeared to be photosynthetically competent, it is possible the xylem and phloem limited flux to low rates. Based on this observation we suggest in future trials that the 'sister' (non-grafted) branches of the understock 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.

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

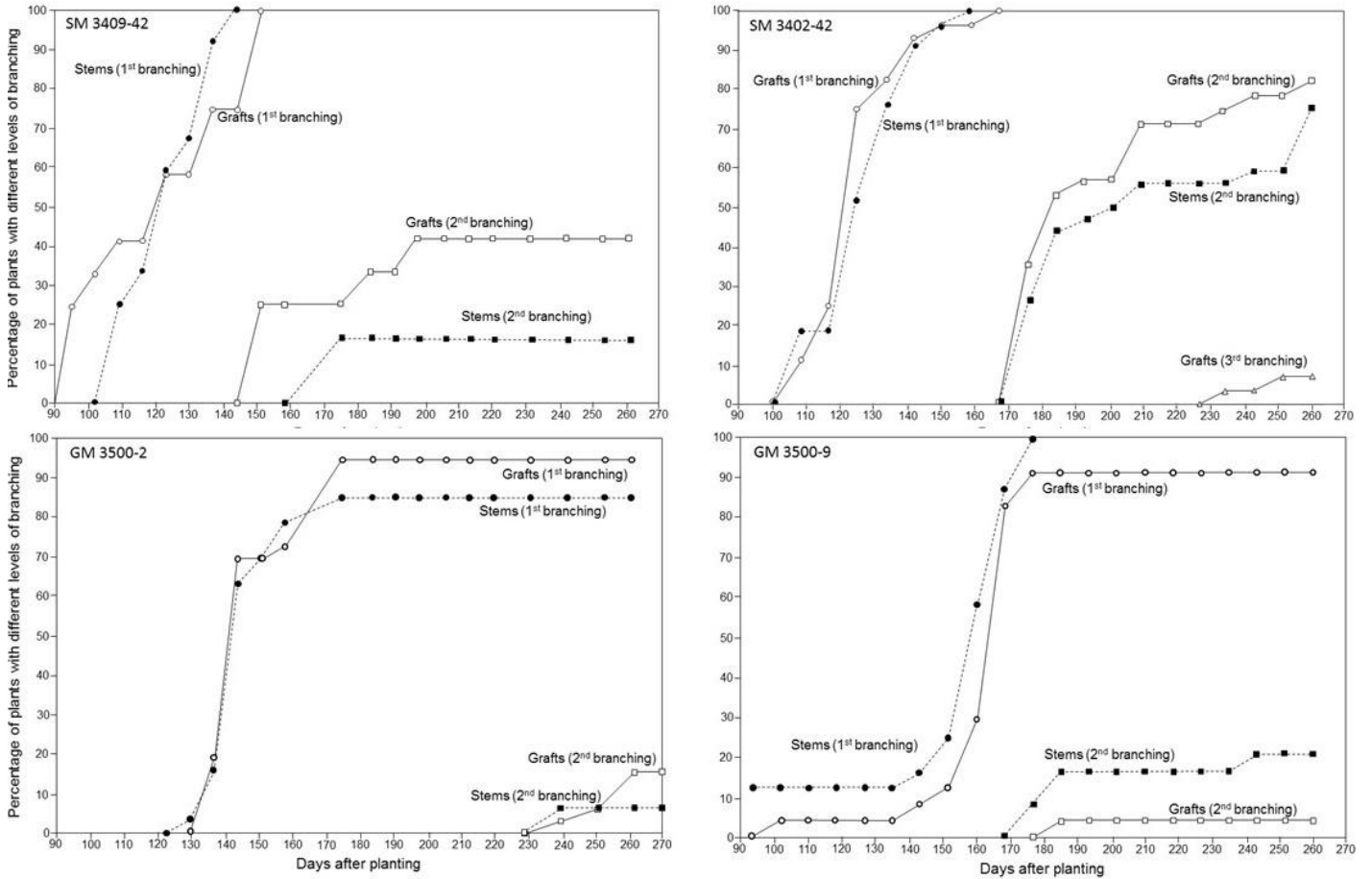


**Figure 3.** Photographs taken on January 4 (174 days after planting). Top photographs illustrate branching without flowering (or perhaps remnants of a rudimentary one). Bottom photographs were taken in plants from grafted cuttings of genotype SM3348-29, with inflorescences at different stages of development.

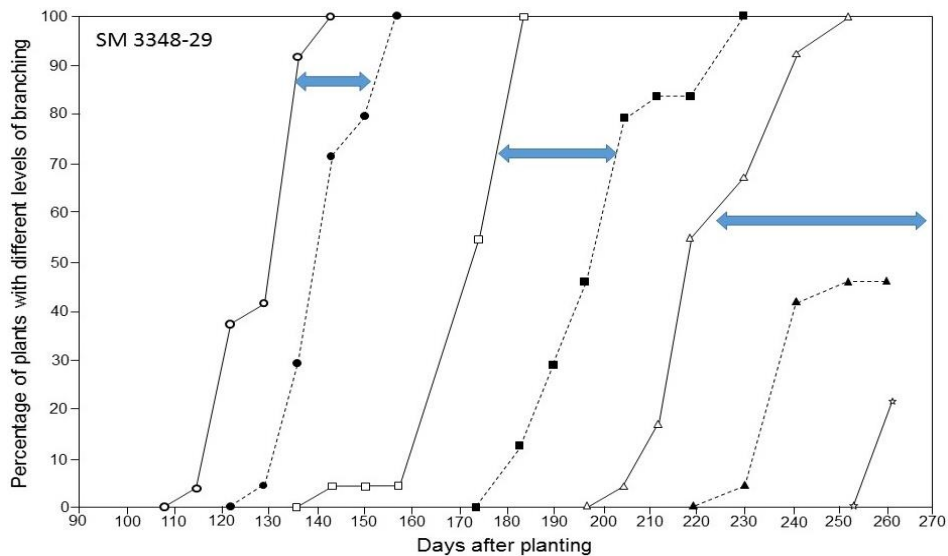
observation. At every branching event, plants from grafts were earlier than those from the non-grafted counterpart. Moreover, the tendency accentuated with each branching event (double-end arrows in Figure 5).

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.

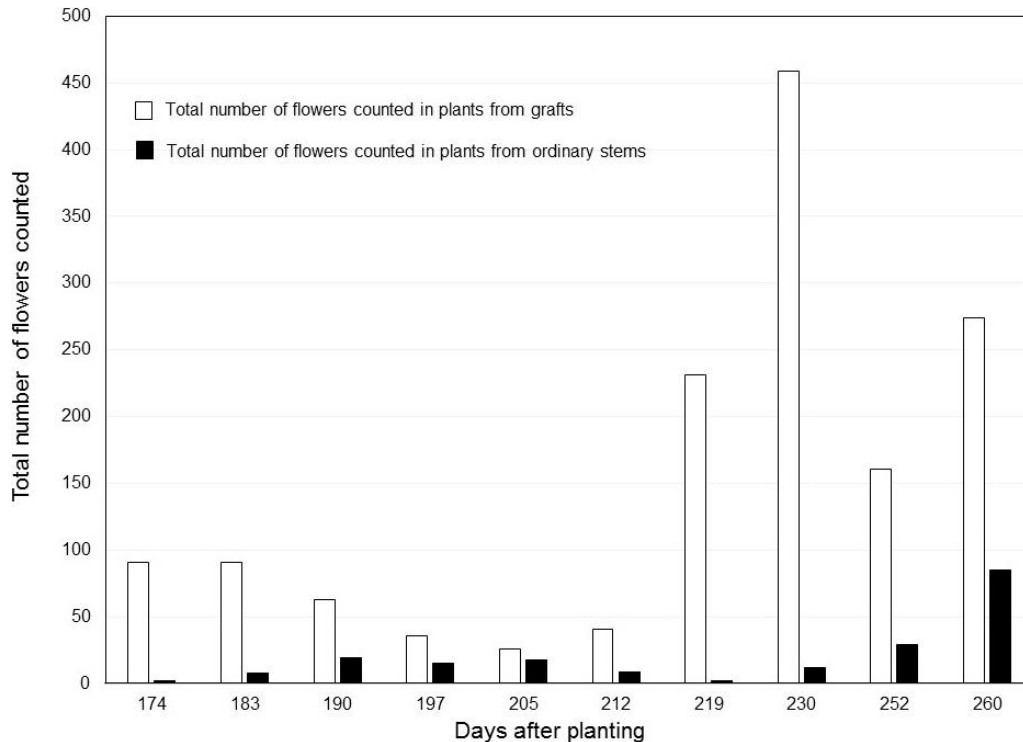
Differences in the number of flowers related to the second branching event are probably more relevant to breeding programs. On February 18 (219 days after planting) plants from grafted cuttings had clearly initiated a second flowering event (231 flowers), which reached a peak few days later (459 flowers). 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.

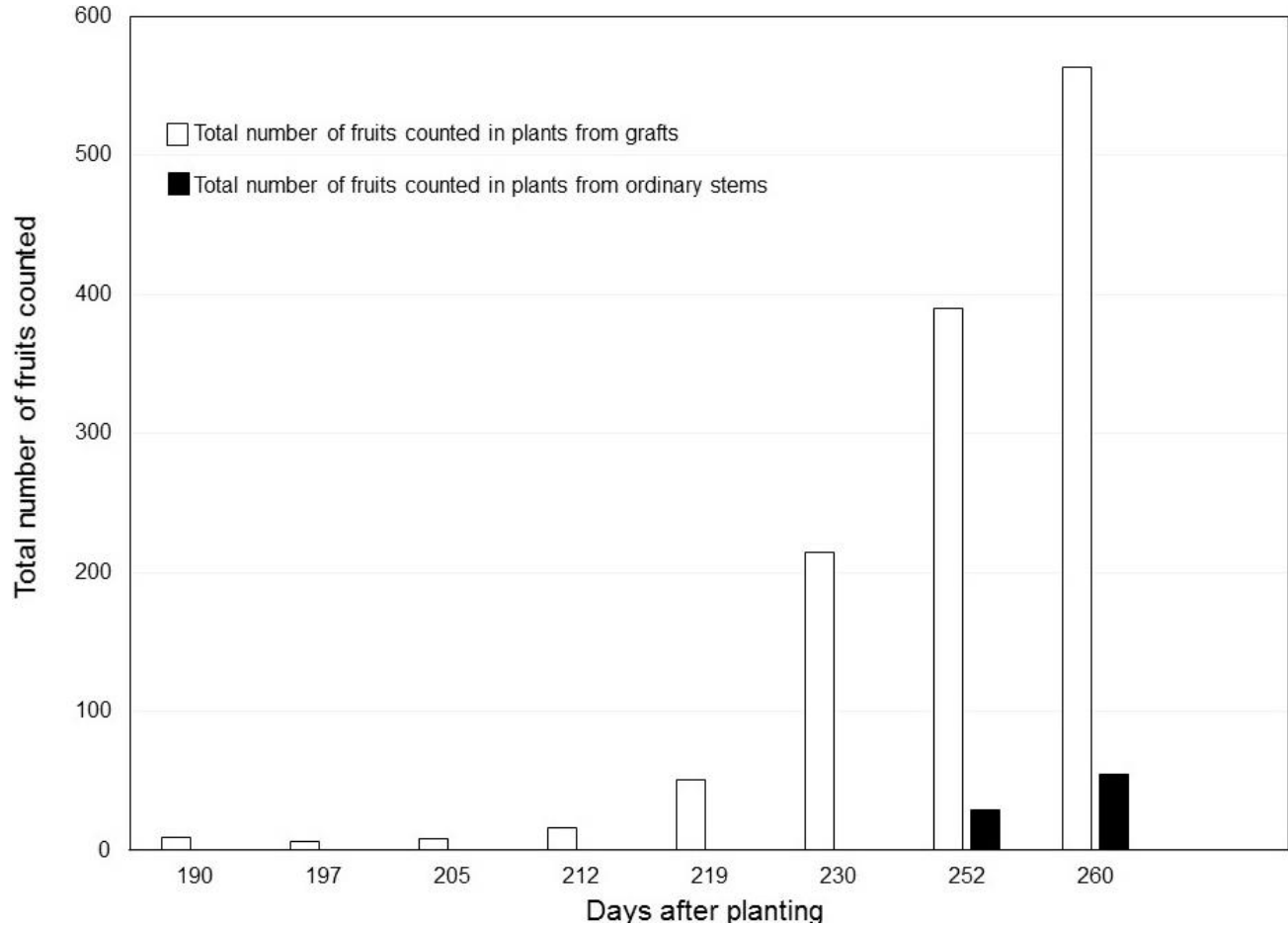
first, second and third branching events (peaks at 174-183; 230 and 260 days after planting). In the first flowering peak 22 out of 24 plants had flowered. In the second peak, 15 plants were bearing flowers. In the last flowering peak, 20 of the 24 plants had flowers. There was some variation in flowering 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 agrees 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](http://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.

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