INDUCTION OF FLOWERING IN CASSAVA

Agreement No. 67724-10566 (under prime agreement No. OPP1048542)
Final Report

1. Introduction

The original aim of the research project was to develop a protocol that will promote early and simultaneous flowering in different cassava genotypes in a crossing nursery. Field visits to Guangxi Subtropical Crops Research Institute (GSCRI) in China suggested unprecedented progress had been achieved there accelerating and increasing seed production in crossing nurseries. The rationale was that the protocols developed at GSCRI could contribute to the overall objectives of the NextGen project. The International Center for Tropical Agriculture (CIAT) would coordinate the linkage between NextGen initiative and GSCRI and also to carry out new research in the Palmira (Colombia) experimental station. Specific activities envisioned at the signing of the Agreement between Cornell University and CIAT included: ***i)*** Further exploration of nutrition basis of cassava flowering in Guangxi province; ***ii)*** Exploration of effective induction methods (techniques) for cassava flowering; ***iii)*** Exploration of effects of different flowering induction technologies & other factors on quality of cassava flowering; and ***iv)*** Exploration of basic elements (conditions) for cassava flowering.

***Expected Output*:** Development of an integrated protocol for the efficient induction of flowering in China, Colombia, and Africa on different cassava genotypes.

2. Subcontract with Guangxi Subtropical Crops Research Institute (GSCRI) in China

2. 1. Formalizing a research agreement between CIAT and GSCRI

A key objective of this grant was to incorporate knowledge and technologies developed at GSCRI for the induction of flowering in cassava. CIAT and Cornell University signed a research agreement at the end of April 2016. However, it was particularly difficult for GSCRI to open an international bank account to receive the funds that the project has to transfer. Only by mid-August 2016 this final requirement could be finalized. Only by September 28, 2016 all the required paperwork could be signed and the collaboration between CIAT and GSCRI formalized.

2.2 Technical report from GSCRI

In March 2017 GSCRI submitted a technical report summarizing the main strategies to induce flowering at high latitudes (for cassava standards). Six clones (SC5, SC9, SC124, SC205, NN199 and XX048) were initially planted in one location by October 2016 (about six month before normal planting time and just before winter). In March 2017, SC5, SC9, SC205, GR891, GR911 and NZ19 were planted (four new varieties compared witht the earlier planting)

One key (and unfortunate) requirement for the protocol of induction of flowering used in GuangXi is that plants need to flower (e.g. branch) to initiate the induction. Therefore, the system does not induce earlier flowering but rather promote fruit set once flowering has occurred. The first action in the process is the removal of branches as soon as their presence can be visually detected (Figure 1). Pruning of branches takes place around March-April (in the plants planted in October the previous year). The second action is the application of undisclosed chemicals and fertilizers by spraying individual plants to control vegetative growth and promote flowering. Simultaneous availability of female and male flowers first occurred in June. At that time, therefore, controlled pollinations could be made.

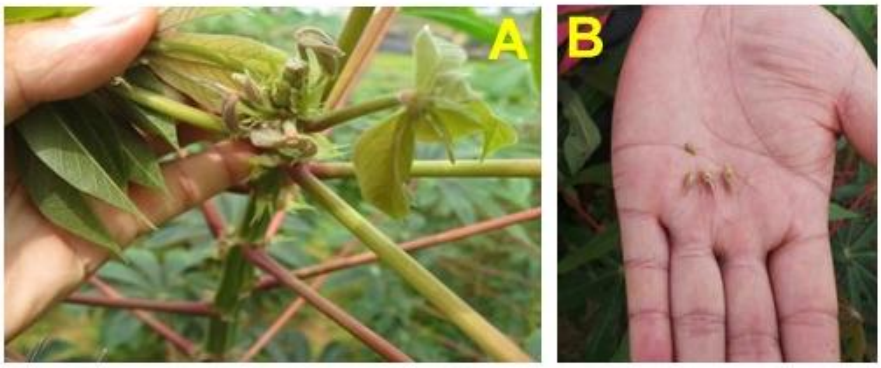


Figure 1. Removal of branches as soon as their presence is detected. **A**. Illustration of a shoot where branches can be identified. **B**. Illustration of removed branches from the apical shoot.

Another important characteristic of the protocol is that plants used for flowering are kept in the field year after year by a ratooning technique. It is clear that best results were obtained when removal of reproductive branches took place on March 10 or before. In these cases 100% of the plants had flowered by June 1st. Whereas only 28% of check plants had flowered at that time. Planting in the spring did not result in positive results with only 4% of the plants flowering by June 20. Ratooning plants is a key activity because flowering induction gets reinforced if the plants are kept in the field. Since the protocol implies removing the branches that plants eventually produce (Figure 1), the flower racemes remain in the apical top of the plant, which can no longer grow (Figure 2). Fruits are harvested before the onset of winter time and, to prepare for a new cycle of flowering, the standing plants are pruned as illustrated in Figure 3.

Figure 2. Illustration of the unusual apical positioning of large inflorescences after the removal of the reproductive branches in two different cassava clones.

Figure 3. Growth of plants in crossing blocks. **A**. Pruning (ratooning) plants in February to promote a vigorous growth in spring. **B**. Alternatively, whole pruned plants may be transplanted at the end of winter (February).

The removal of reproductive branches not only affects the proportion of plants flowering but also in the size and number of flowers produced. The earlier the pruning the higher the number of female flowers counted across the different genotypes. Number of male flowers remain relatively high. The size of raceme was also largest when reproductive branches were removed as early as March 1st. However, there are some differences among genotypes. Development of complete (hermaphrodite) flowers, was fount to be of common. In this type of flowers self-pollinations are feasible and produce seed that can be used in the process of accelerating inbreeding in cassava.

As expected, the abundant and earlier production of flowers allows fruits to develop in relatively large quantities (Figure 4). Since flowering takes place earlier in the year, fruits can be collected before the cold months (December and January).

Figure 4. Illustration of abundant fruit set in two different cassava clones treated with the protocol developed at GSCRI.

In addition to the key steps of earlier planting or ratooning plants and removal of reproductive branches, plants are also treated with a special combination of agrochemicals (amino acid, glyphosate, glucose, humic acid, etc.). It is not clear, however, whether the chemical agents significantly affects the amount and timing of flowering in our experiments.

3. Research conducted at CIAT: materials and methods

3.1 Germplasm

CIAT researchers selected first a group of six contrasting genotypes based on their flowering biology. Some of these clones had been used in a pioneering research using the grafting technique that resulted in a publication (Ceballos et al., 2017):

- An “asparagus” clone with sessile leaves (no petiole) and non-branching (**GM 3893-66**).
- The genotype that did not branch, nor flower from the grafting experiment (**SM 3409-43**).
- A genotype that branched but did not flower from the grafting experiment (**SM 3500-2)**.
- The genotype that branched and flowered earlier in the grafting experiment (**SM 3348-29**).
- A late branching/flowering commercial clone (**CM4919-1**)
- An intermediate branching/flowering experimental clone (**GM 971-2**).

3.2 Location

All experiments were planted at CIAT Experimental Station in Palmira, Valle del Cauca, Colombia. A key characteristic of this location is its high altitude (about 1000 meters above sea level) which results in relatively cool nights.

3.3 Treatments

Three type of treatments were evaluated through the execturion of this project:

- Extended photoperiod or red light district (RLD). This treatment was evaluated two consecutive seasons. In every case the source of red light (peak around 620-640 nm) was LEDs. In the first season 5 or 10 individual LEDs or one or two 20-cm LED tapes per plant were used. For the second season, other sources of red light (e.g. a single 50W reflector) and the effect of night breaks were also considered. Each of the six genotypes listed above was planted in single row plots with 10 plants each and exposed to the different source of red light or duration of the illumination period (for the nigh break assessment conducted during the 2nd season).

- Plant growth regulators (PGR). Three treatments were considered: Silver thiosulfate (STS); Benzyladenine - MaxCel (BA); and a combination of these two PGRs. In addition, a water control (with the addition of tween surfactant which was also used with the PGR solutions) was also included. These treatments were evaluated two consecutive seasons. Each of the six genotypes listed above was planted in single row plots with 10 plants each and sprayed with the different PGR.

- Early pruning of young branches as soon as flowering had been induced. This technique was tested preliminarily only one growing season

The effect of each individual treatment was evaluated, as stated above, in a single row with ten plants each. Each plant was considered an independent experimental unit.

In addition two check rows were planted for each genotype. During the second season RLD and PGR were applied simulatenously to the same set of genotypes in evaluate if a synergistic effect between the two sources of stimuli would result in an even earlier flowering.

4. Research conducted at CIAT: results

4.1 Effect of extended photoperiod in the RLD

RLD resulted in promising results since plants of most exposed to this type of stimulus branched earlier. However, in cassava the first flowering event is usually sterile. Fruit and seed set can be observed only during the second and later flowering events. In two particular clones, however, the earlier branching also resulted in earlier and more aboundant production of flowers and, eventually, fruits and seeds. The experiments were finalized at seven to nine months of age, thus it was not possible to assess if earlier fruit and seed production could be observed in the other genotypes that were less reluctant to respond.

Results from the RLD experiment in the 1st growing season are summarized in Figure 5. For each genotype branching data from plants under extended photoperiod and the respective check (not illuminated at night) is presented. **GM**

971-2 branched several times (even in the check plants). However, first branching of illuminated plants was about 35 days earlier. Similar differences were observed for the second branching. Only some illuminated plants produced a third branching. In **CM 4919-1** results were more contrasting. More than 90% of plants in RLD had flowered around 90 days after planting (DAP), whereas only 40 % of check plants had branched for the 1st time by 150 DAP. More relevant only illuminated plants produced 2nd and 3rd branching, which are the ones related to the production of viable flowers and fruit/seed set. The remaining genotypes also showed a clear induction for earlier branching, with negligible branching in the respective check plants. Figure 6 provides an illustration of plants from two of the clones harvested in the red light district (CM 4919-1 and SM 3409-43). For each genotype, three photos are provided. In every photograph there are two plants. The one on the left is a check plant (dark at night), whereas the plant on the right in each photograph had been illuminated at night. The effect of extended photoperiod is obvious as illuminated plants clearly branched more profusely. Also interesting was the unexpected increase in plant height in the case of CM 4919-1.

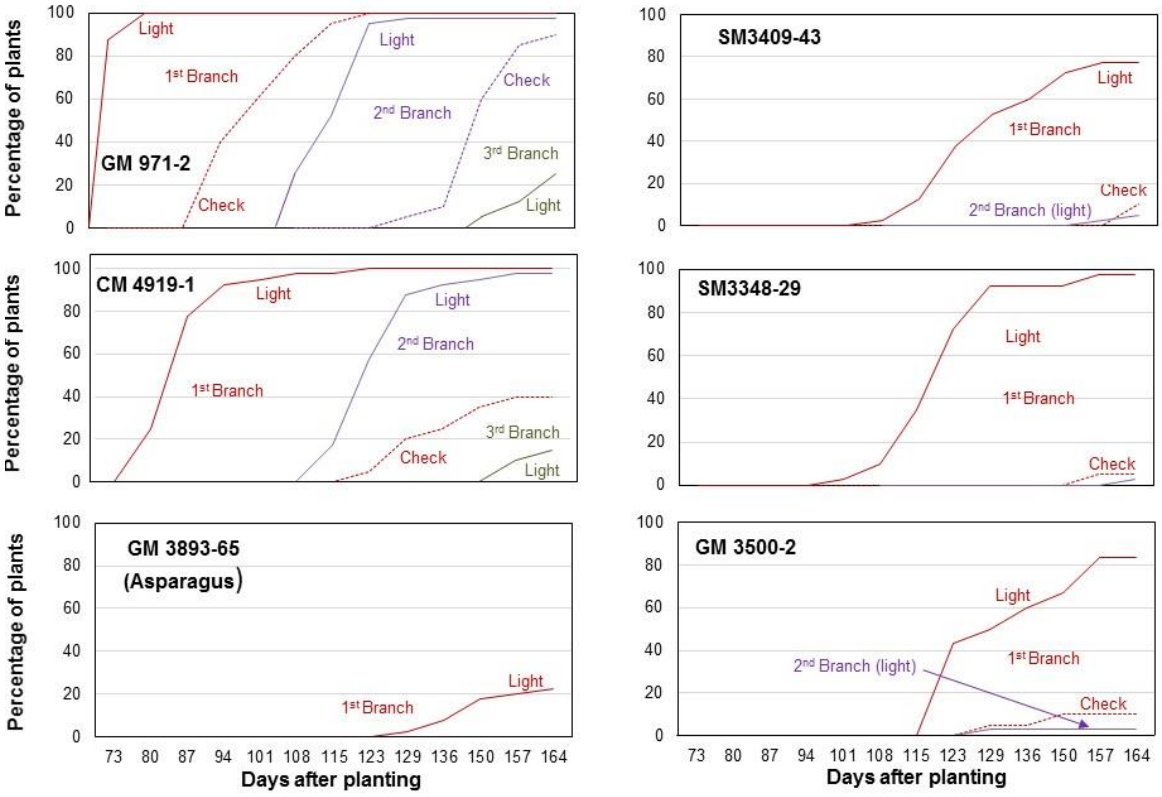


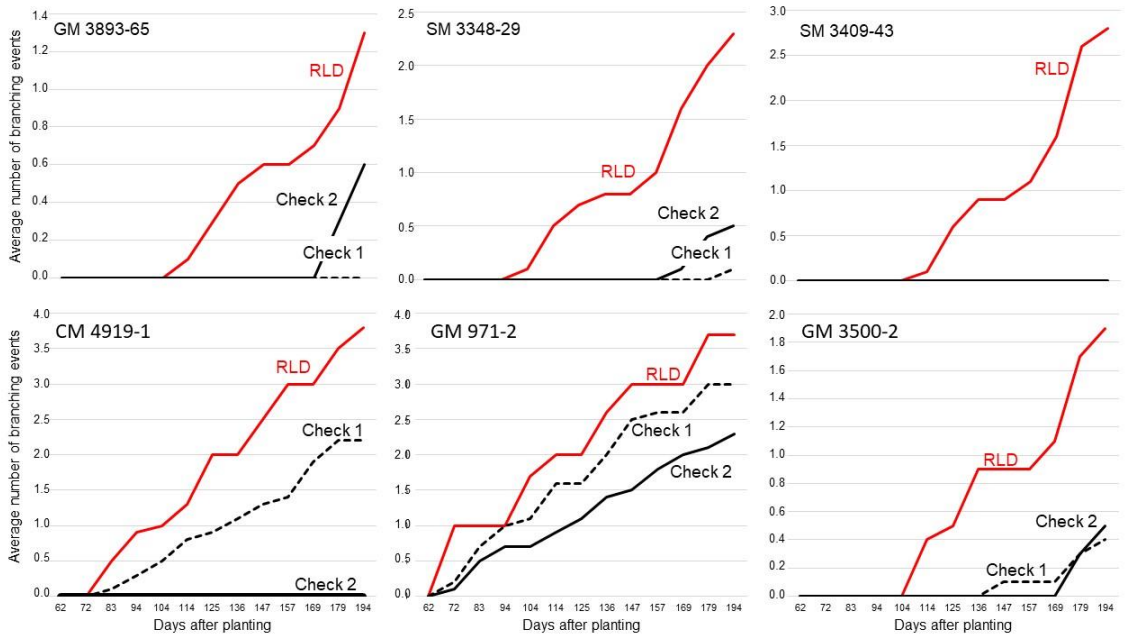
Figure 5. Results for the induction of flowering in the RLD. **GM 971-2** is an early flowering genotype; **CM 4919-1** is a late flowering genotype; GM 3893-66 ("*asparagus*" cassava) does not flower in less than a year; **SM 3409-43** was selected from the grafting experiment (did not branch, nor flowered); **SM 3348-29** branched and flowered in the grafting experiment; **SM 3500-2** branched but not flowered in the grafting experiment.

Figure 6. Results for the induction of flowering through extended photoperiod. Contrast between check plants (left plant in each photo) and illuminated plants from **CM 4919-1** and **SM 3409-43**. The effect of photoperiod extension is obvious as in every case, plants on the right of each phot had branched at least once, whereas those on the left had not branched in any case.

The same experiment described above was planted for validation during a 2nd season. Plants were illuminated with red lights from five individual LEDs all night long. LEDs were placed about 10-20 cm above the growing tip of the plants. As plants grew, the position of the LEDs were periodically moved up to maintain that target distance. Therefore thre was only one light intensity considered during this second evaluation. The remaining LEDs used during the 1st season were used for the night break experiments. By and large the results from the 2nd season confirmed earlier findings. In every case, plants growing under extended photoperiod conditions (RLD) branched earlier and more profusely than the check plots (**Figure 7**). The response variable used in most of this report is the average number of branching events per plant. An average of 2.5 for a given genotype implies that half of the plants had branched two times, whereas the remaining half of the plants branched three times.

Interestingly, there was a sharp difference between the checks, particularly of CM 4919-1 and GM 3893-65. In th case of CM 4919-1 the first check plot branched considerably, whereas no branching occurred (as expected) in the second check plot. CM 4919-1 normally flowers for the first time about 8-9 months after planting (MAP). In the case of GM 3893-65 (“asparagous” cassava), which usually flowers when plants are more than a year old, six plants in the second check plot branched about six MAP. No plant in the first check plot of this genotype branched or

flowered (as expected). It is suspected that personnel inadvertently collected planting material of the genotypes to be used as checks from plots that had been grown under RLD conditions the previous season. As demonstrated below, a memory (or residual) effect of the flowering induction is transmitted to the following generation.



Figue 7. Average number of branching events per plant in six different genotypes. For each clone one row was under extended photoperiod conditions with five LEDs maintained about 10-20 cm above the growing shoot. There were two check plots as well. Data from the first checks (particularly for C 4919-1) are supiciuous.

4.1.1 Exploration of the impact of night breaks on the induction of flowering

Six different night break lengths were evaluated (30, 60, 90, 120, 180 and 240 min) on two genotypes (CM4919-1 and GM 971-2). Breaks took place around 12 PM (e.g. the 30´ break began at 11:45 PM and ended at 00:15 AM). Previous experiences suggested that light intensity (when plants were illuminated all night long) did not have any effect on plant responses. Different sources of light were used for the different lengths of nigh breaks. A maximum light intensity (two 20cm LED tapes) was used for the 90 and 240 min night breaks; an intermediate light intensity (one 20cm LED tape) was used for the 30 min night break; and a low intensity illumination (10 individual LEDs) was used for the 60, 120 and 180 min night breaks. For the all-night long RLD plants were illuminated with the lowest intensity (only five LEDs). There was, therefore, a confounding effect between duration of night break and light intensities.

Figure 8 presents the results from the two clones evaluated. The same information from the check plots presented for the previous experiments was used also here.

Therefore, the differences in the two checks, particularly from CM 4919-1, are again observed. There was no clear cut response for the intermediate flowering clone GM 971-2 (left plot in Figure 8). However, an interesting observation could be made. There were two treatments that did not show a better response in comparison with the second check, which probably provides the most reliable information. These treatments are the 60 and 120 min. These are two of the three treaments exposed to low light intensity. In fact, the third night break treatment with lowest response was the third rwo in which low light intensity had been used (180 min). The best three responses were observed (independently on duration of the night break) in those cases with intermediate to high light intensity.

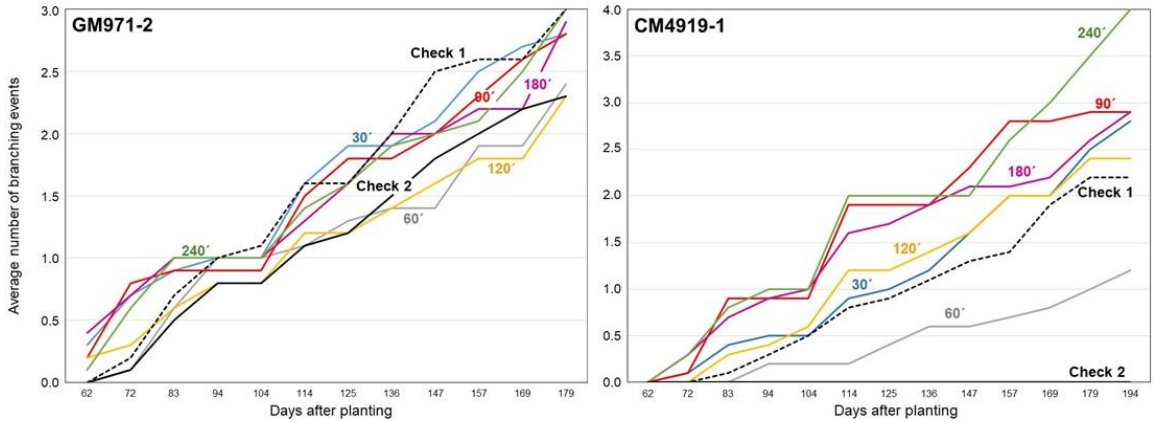


Figure 8. Impact of nigh breaks of different durations (from 30 to 240 minutes) on the two clones selected. For treatments 60, 120 and 180 min, low light intensity was used. For the 90 and 240 min treatments, a high light intensity was used. In the 30 min treatment, plants were illuminated with an intermediate light intensity.

In the case of CM 4919-1 (right plot in Figure 8), responses to the nigh break were much clearer. All treatments (except the 60 min) were better than the two checks. The 60 min treatment was the shortest (time wise) using low intensity light. Results from this experiments show that night breaks are indeed useful inducing earlier flowering. This is important in the case a “portable” illumination system based on solar panels is used. In that case, the amount of energy available may become a bottleneck. This experiments was also interesting because it allowed detecting a clear quantitative response to the stimuli. The longer the night break and/or the higher the light intensity, the better the response from the plants. This quantitative response could not be observed the previous season as different light intensities were used but throughout the night.

4.1.2 Definition of the minimum light intensity to induce flowering in cassava.

Another experiment was planted during the 2nd season to test the usefulness of 5m long LED tapes that were kept at a fixed distance from the ground. In one case, there were four rows and the LED tape was placed between the two central rows at 80 cm from the ground. In a second plot, five rows were planted and the LED

tape was placed at 100 cm from the ground immediately above the central row. Each row had 10 plants, five from GM 971-2 and the remaining five with CM 4919-1. In addition to confirming the response of the plants to the light generated by the tapes, the experiment allowed a gradient of light intensity higher in the internal rows, compared with the external rows. A key feature of these plots is that lights were kept in a constant positioning, thus greatly simplifying the implementation of the experiment.

Figures 9A and **9B** present the results from the two plots. In addition to the responses to the LED tapes, these figures also provide the response for the same genotypes presented in Figure 1 ("*Lights on top of the plants*"). Data shown in Figure 1, the lights were low intensity (5 individual LEDs) but were kept at a 10-20 cm distance from the growing shoot of the plant.

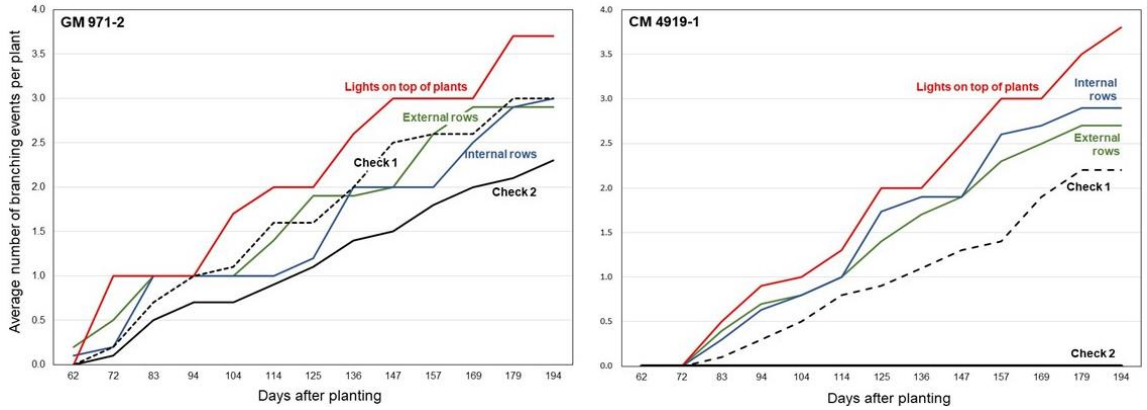


Figure 9A. Response observed in a plot with four 10-plant rows (half with GM 971-2 and half with CM 4919-1) illuminated with a 5-m long LED tape positioned between the two internal rows at fixed 80 cm from the ground.

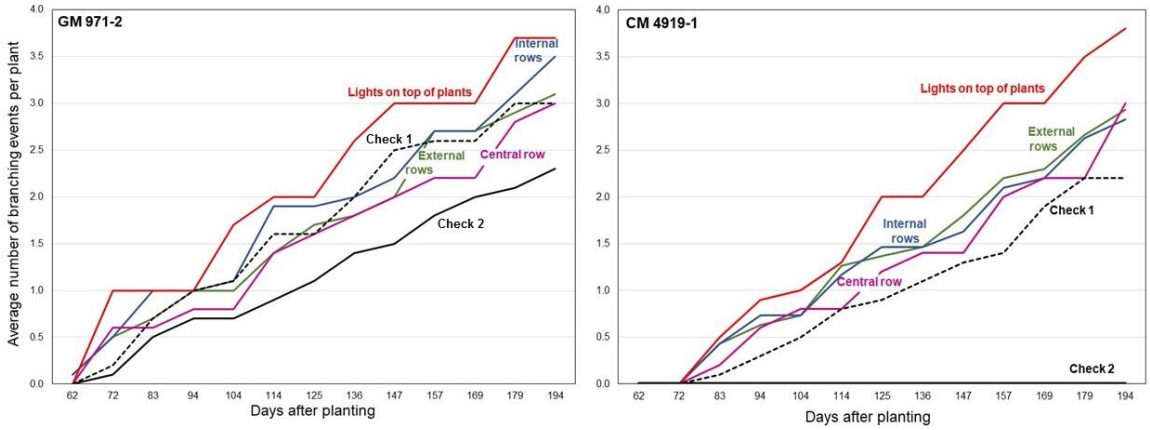


Figure 9B. Response observed in a plot with five10-plant rows (half with GM 971-2 and half with CM 4919-1) illuminated with a 5-m long LED tape positioned on top of the central row at fixed 100 cm from the ground.

Results presented in Figure 9A and 9B are consistent with results from the experiments described above. Illuminated plants showed an earlier branching than the most reliable check (# 2). The best responses were observed from plants where the lights were placed 10-20 from the top and moved up as the plants grew (lights on top of plants). In the case of CM 4919-1 the response of plants in the two internal rows was, as expected, better than those in the external rows (**Figure 9A**), but that was not the case for GM 971-2. Differences, howerver, were not large. In the five-rows plot a similar trend was observed (Figure 9B): the best treatment came from lights on top of the plants, followed by plants illuminated with the LED tape and then check # 2. There were, however, no clear differences between plants in different rows within the experiment. It is clear that the illumination of plants in these two plots was above the threshold required to elicit a response which was, nonetheless, lower than that of the lights placed immediately on top of the plant.

The best experiment to assess the light intensity required to elicit a response of plants to illumination with red light during the night was based on the use of a 50W reflector. The experiment included ten rows with a spacing of 1.5m. Each row was 13.5 m long and half of them was planted with 14 plants from GM 971-2 and the other half with 14 plants from CM 4919-1. Spacing of plants within the row was 50 cm. The reflector had a fixed position 3m above the ground on top of the center of the plot (**Figure 10**). In this experiment, there is a clear gradient in light intensity (which is related to the inverse of the square of the distance to the source). This gradient, however varied as the plants grew. For those plants closer to the center of the plot, light intensity increased as they grew closer to the source. However, for those plants in the periphery of the plot the top of the plant would grow out of the "illuminated cone". Plants at the very periphery of the plot, as expected, branched later than those closer to the center. There was a threshold area (last 2-3 plants in the row, and first and last row in the plot) in which plants flowered at about the same time as the check # 2 (which was planted nearby). There were a group of plants (10^{th} to 12^{th} within each row and the second and the row before the last in the plot (Figure 5) that received about 0.01 µmol m^{-2} s^{-1} at planting time. This very low threshold was enough to elicit already an earlier flowering on both gentoypes (compared with the checks). Plants closer to the center (receiving > 0.03 µmol m^{-2} s^{-1}) flowered even earlier with not much diference among them. The light intensity immediately below the source of light at ground level (e.g. 3 m) was 0.1 µmol m^{-2} s^{-1}.

Conclusions from extended photoperiod experiments

- Most genotypes responded favorably (e.g. branched earlier) under RLD conditions
- A very low light intensity is enough to trigger responses. However, when night breaks (rather than all-night illumination) are used, light intensity has an effect suggesting the need for a minimum threshold in the amount of light received by plants at night

- Because of the short duration of the experiments not all plants branching earlier had the time to also produce fruits and seeds.

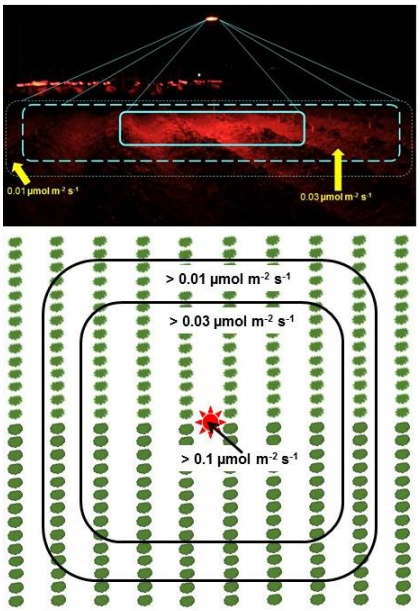


Figure 10. Evaluation of the 50w reflector as source of red light. The reflector had a fixed position 3m above ground. Two genotypes (CM 4919-1 and GM 971-2) were used. In the periphery plants received less than 0.01 µmol m^{-2} s^{-1} of light, which seems to the the threshold to elicit a response from the plants.

4.2 Validation of the effect of plant growth regulators

PGR experiments yielded positive results as well. Our results suggested that the best responses were observed after the combined application of benzyladenine (BA) and silve thiosulfate (STS). The same genotypes used in the red light district were also employed for this experiment. Spraying (see Figure 11) was initiated 75 DAP and at weekly intervals thereafter. As expected, there was some phytotoxic, but manageable effect on some of these treatments.

As in the case of extended photoperiod, there were interesting and genotype-dependent results after the application of plant growth regulators. Figure 12 presents the results of the 1st season of evaluations. Most remarkable is the possibility to count dozens of fruits in the "asparagus" genotype (GM 3893-65) as early as five months after planting. Also interesting was the fact that fruits could be obtained from the first flowering event, which is usually sterile. In general, the best results were observed after the combined application of STS and BA. It should be pointed out that plants were harvested at 7.5 months (August 8 through March 20) after planting and therefore counting of fruits in the third branching event was very limited as it had just happened and only in few clones.

Figure 11. Illustration of the methodology employed in the application of the plant growth regulators.

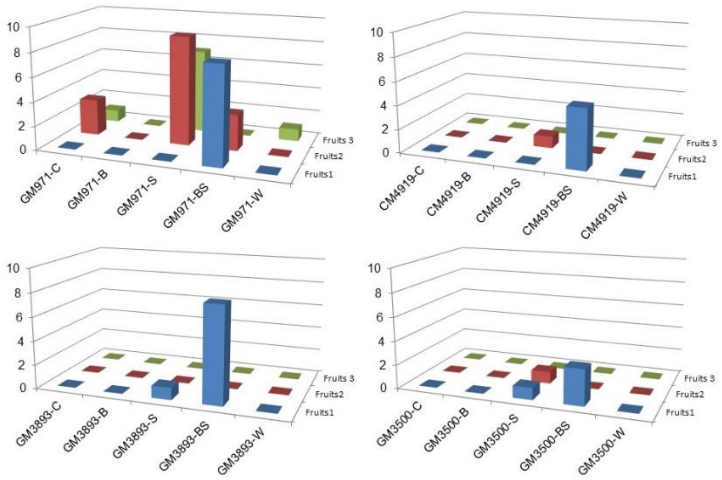


Figure 12. Response of four genotypes to the application of plant growth regulators (number of fruits in the three levels of branching (Fruits 1, 2 and 3, respectively). The name of each genotype is followed by a code standing for the check (-C); application of benzyladenine (-B); silver thiosulfate (-S), both regulators (-BS), and water+ tween 20 (-W).

The same experiment in the 1st season was planted for validation during the 2nd season using the same set of plant growth regulators (PGR): Silver thiosulfate (**STS**), Benzyladenine - MaxCel (**BA**) and combination of both (**BA+STS**). Because of the results from the previous season indicaged that a treatment with water and Tween 20 did not elicit any positive or negative reaction in the plants, this treatment was not repeated during this second season. Spraying (as done in the 1st season) was initiated 75 days after planting (DAP) and at weekly intervals thereafter. For some reason the phytotoxic effects observed during this second

season were stronger that previously observed. In addition, some plots were affected by ants that, in a matter of two days defolitated few plots. The plots for evaluating the effects of PGR were located at the end of the rows and tended to be affected more by occasional water logging as this was the area of land where water drained the last. Plant growth of these plots was not as vigorous as that in other experiments for the induction of flowering described in this report.

Figure 13 summarizes the results observed during this second season evaluating PGRs. In general results suggest (as was the case in the previous season) that combining the two PGRs provided the best responses (exept for GM 3500-2 and SM 3409-43). In several cases, as exsplained above, the checks branched earlier than expected. This is, for example the case of GM 3893-65 which generally branches when plants are older than a year. In this experiment, however, the second check flowered as soon as six MAP. It should be emphasized that the only genotype that would naturally flower for the first time about 3-4 MAP is GM 971-2. The five remaining genotypes would not flower within six MAP.

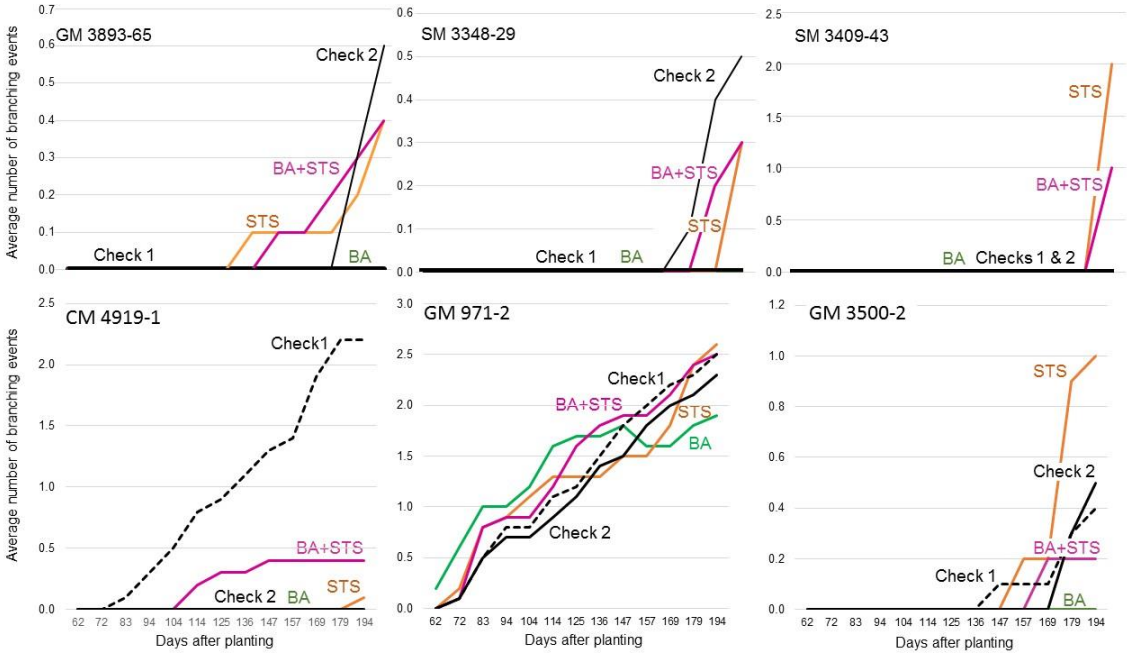


Figure 13. Average number of branching events in ten-plant plots of six different genotypes treated with three different plant growth regulators. In some cases the two checks show very contrasting performances probably because the planting material for one of them was taken from plants that in the previous season had been exposed to stimuli to induce flowering.

As illustrated in Figure 13, however, plants treated with PGR began flowering much earlier. In the case of CM 4919-1 between 3 to 4 MAP. GM 3893-65, the “asparagus” cassava began flowering 4-5 MAP. Both checks of GM 3500-2 began

flowering around 5-6 MAP (about 40% of the plants), whereas all plants treated with STS had branched by the end of the experiment. SM 3348-29 and SM 3409-43 should not flower as was the case for one of the two check plots. Plants of these two clones treated with STS alone or combined with BA, began flowering six MAP. In general, plant responses to the application of BA alone were disappointing.

Conclusions from extended photoperiod experiments

- Most genotypes responded favorably (e.g. branched earlier) in response to PGR treatments
- The best response were generally observed in the combination of STS+BA
- Genotypic resonses to PGR were observed
- Phyto-toxic effects varied in the two consecutive seasons the experiments were conducted

4.3 Combination of photoperiod extension and plant growth regulators

The same six experimental clones used for RLD and PGR experiments were evaluated in separate plots in which plants were grown under RLD conditions since planting and then treated with the three PGR described above. Each genotype was planted in 10-plant rows. Application of PGR began in 5 of the plants 75 DAP, and 65 DAP in the remaining 5 plants. The main objective of this experiment was to asses if the combination of both stimuli promoted earlier flowering compared with a single stimulus response, or else, if stronger effects could be achieved in “shy” genotypes such as SM 3348-29 and SM 3409-43. Results, however, were disappointing and no clear advantage could be observed combining the two sources of stimulus.

4.4 Validation of the pruning of young branches for early fruit and seed set

The involvement of CIAT within the first phase of the NextGen project was to work together with GuangXi Subtropical Crops Reasearch Institute (GSCRI) in Nanning. Personnel from this institute had developed an interesting technology to induce flowering in that region of China. Cassava faces a limitation to produce seeds because of the relatively short growing season at that latitude. In August 2017, Hernán Ceballos visited GSCRI and learned the basic technology used to induce earlier flowering. In fact it is a technology that does not induce earlier flowering but rather allows viable fruit and seed set from the first flowering event, which under normal circumstances is sterile. A key feature of the protocol relies on the early detection of the apical meristem shift from vegetative to reproductive growth. The vegetative shoot has a tear shape, whereas the early reproductive shoot acquires a globular shape (**Figure 14**). Soon after this shift the emergence of young branches can be detected and as soon as this is feasible they should be removed (**Figure 14**). It is possible that by pruning young branches the inflorescence assumes the apical dominance allowing fruit and seed set in the first inflorescence.

Only one cultivar (CM 4919-1) was planted in five rows with ten plants each. On top of the first row a 5m long LED tape was positioned. The first row, therefore received a high light intensity which decreased gradually in rows farther away from it. The impact of flowering time (e.g. average number of branches per plant) gradually reduced, particularly for the 5th row. CM 4919-1 typically produces three branches at each flowering event. Some of the young branches were pruned, as soon as they could be distinguished but in the 2nd flowering event (rather than in the 1st as made in China). Therefore, there were three shoots/plant that could be pruned after the apical meristems shifted to flowering (in the 2nd flowering event). Some shoots were left unpruned while others were pruned for comparison sake. This was done randomly.

There was a positive response in inflorescences from pruned shoots as they grew larger with higher number of flowers and enhanced fruit set (**Table 1, Figure 15D**). A total of 50 plants were planted and 47 were available for this experiment. A total of 191 apical meristems were available from these 47 plants and 122 of them were left untouched while the remaining 69 were pruned. Decision to prune or not was random and, to a some extent, determined by the early identification of young shoots that had already shifted towards reproductive growth. Table 1 summarizes the result of this experiment. The number of fruits and seeds basically doubles in shoots that were pruned versus those that were not.

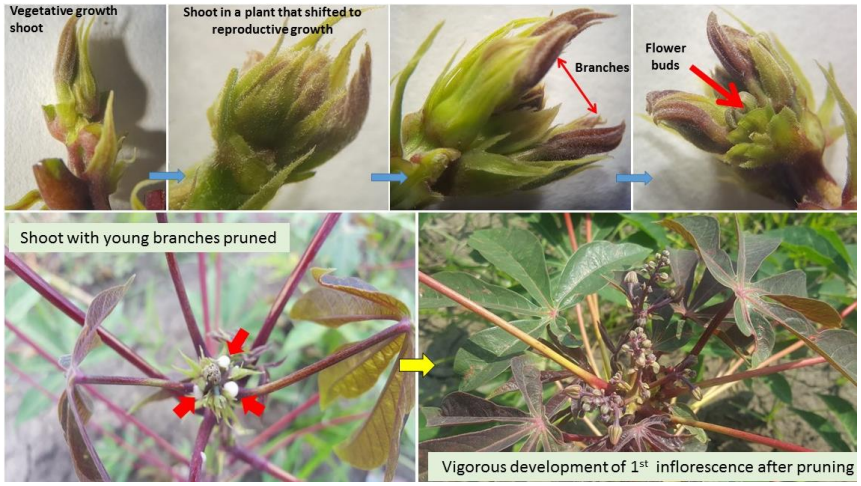


Figure 14. Illustration of the shape change in the apical shoot as it transitions from the vegetative into the reproductive mode (top photographs). Pruning of young branches results in the inflorescence (rather than the branches) excerting the apical dominance.

Table 1. Average number of fruits and seeds per apical meristem in pruned or not pruned branches. Data presented for each of the five rows of the experiment. A LED tape with red light was placed immediately above the first row. Plants in rows further away received less intense illumination showed fewer number of branches.

Row	# of plants	Average # of branches per plant	Non pruned		Pruned	
			Fruits (#)	Seeds (#)	Fruits (#)	Seeds (#)
1st	9	4.89	0.22	0.39	0.50	1.13
2nd	10	4.40	0.74	1.70	1.29	3.24
3rd	9	3.78	0.59	1.32	0.75	1.17
4th	9	4.44	1.00	2.40	1.69	3.69
5th	10	3.50	0.85	1.35	1.33	2.47
Across the five rows			**0.61**	**1.27**	**1.22**	**2.61**

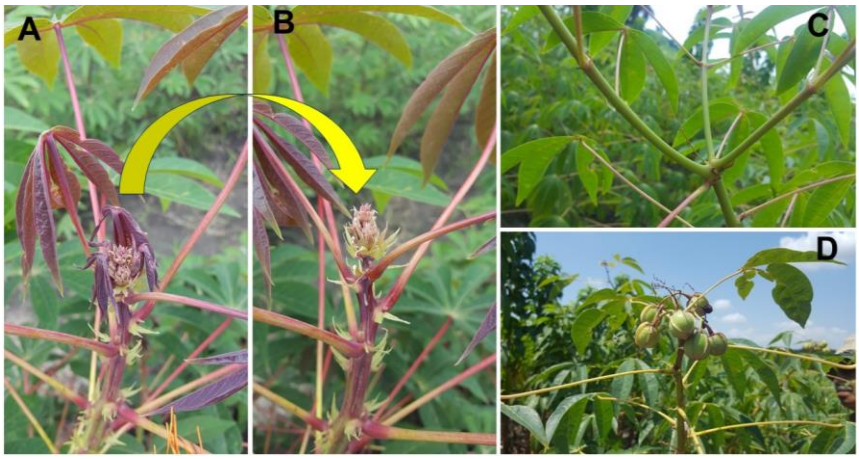


Figure 15. Photographs illustrating the pruning of young branches in cassava soon after flowering can be detected. **A.** An apical meristem in which flowering has been detected soon after it was initiated; **B.** The same shoot after pruning the young branches; **C**. An example of an inflorescence that do not set fruits (frequent in early flowering events); **D**. The effect of pruning on the appearance of to top of the plant showing good fruit set.

Preliminary conclusions from the pruning technique

- The technique proved to work in conditions different from those in China
- The technique does not need the addition of chemical/fertilizer treatment different from those used for standard cultivation of cassava
- Pruning young branches soon after induction of flowering promotes fruit and seed set even in the 1st branching event
- Pruning requires that flowering had been previously induced. Its main advantage would be in combination with RLD or PGR

4.5 Assessment of the residual effect of the induction of flowering

It has been proposed (and earlier interactions with GSCRI supported the idea) that induction of flowering could have a lasting effect if stems from stimulated plants were used as source of planting material. Stems from the two most responsive genotypes the previous season (GM 971-2 and CM 4919-1) were collected and cuttings planted during the present season. Results of this evaluation are presented in **Tables 2** and **3** for genotypes GM 971-2 and CM 4919-1, respectively.

In the case of GM 971-2 no plant branched for a second time on check plants, whereas a considerable number of plants derived from “stimulated” stems branched twice and produced flowers. Production of fruits, however, could not be followed as the experiment was harvested as flowering had just began to occur. Not all stimuli had an impact of the performance of the respective “progeny” but enough differences were observed to demonstrate that, indeed, there is a memory effecto. Results from CM 4919-1 were not as clear as was the case for GM 971-2. Plants from cuttings obtained from BA or BA+STS branched twice and produced flowers in this second branching event. In no case, however, plants produced fruits.

Table 2. Results of the experiment to assess the residual effect of flowering induction stimuli through the use of planting material from the stems of treated plants of genotype GM 971-2.

Clone	Treatment of plants from which stem cuttings were taken		Percentage of plants					
			First branching			Second branching		
			Branch	Flowers	Fruits	Branch	Flowers	Fruits
GM 971-2	Check 1		66.7	0.0	0.0	0.0	0.0	0.0
	Check 2		20.0	0.0	0.0	0.0	0.0	0.0
	PGR	BA	50.0	0.0	0.0	40.0	30.0	0.0
	PGR	STS	55.6	0.0	0.0	22.2	0.0	0.0
	PGR	BA+STS	50.0	0.0	0.0	12.5	0.0	0.0
	PGR	Water	0.0	0.0	0.0	0.0	0.0	0.0
	RLD	5 LED	75.0	0.0	0.0	50.0	25.0	0.0
	RLD	10 LED	80.0	0.0	0.0	40.0	40.0	0.0
	RLD	1 Tape	100.0	0.0	0.0	0.0	0.0	0.0
	RLD	2 Tapes	28.6	0.0	0.0	0.0	0.0	0.0

Table 3. Results of the experiment to assess the residual effect of flowering induction stimuli through the use of planting material from the stems of treated plants of genotype CM 4919-1.

Clone	Treatment of plants from which stem cuttings were taken		Percentage of plants					
			First branching			Second branching		
			Branch	Flowers	Fruits	Branch	Flowers	Fruits
CM 4919-1	Check 1		0.0	0.0	0.0	0.0	0.0	0.0
	Check 2		22.2	0.0	0.0	0.0	0.0	0.0
	PGR	BA	30.0	0.0	0.0	10.0	10.0	0.0
	PGR	STS	0.0	0.0	0.0	0.0	0.0	0.0
	PGR	BA+STS	10.0	0.0	0.0	10.0	10.0	0.0
	PGR	Water	0.0	0.0	0.0	0.0	0.0	0.0
	RLD	5 LED	Not available					
	RLD	10 LED	0.0	0.0	0.0	0.0	0.0	0.0
	RLD	1 Tape	0.0	0.0	0.0	0.0	0.0	0.0
	RLD	2 Tapes	10.0	0.0	0.0	0.0	0.0	0.0

5. Scientific publications and presentations

Ceballos, H., J.J. Jaramillo, S. Salazar, L.M. Pineda, F. Calle and T. Setter (2017). Induction of flowering in cassava through grafting. Journal of Plant Breeding and Crop Science 9:19-29.

The following posters were presented during the GCP21 conference (Cotonou, Benin. June, 2018) and ISTRC (Cali, Colombia October 2018):

1. L. Marcela Pineda, Nelson Morante, Sandra Salazar, Peter Hyde, Tim Setter, and Hernán Ceballos (2018). Induction of flowering I: photoperiod extension through a red lights district.
2. L. Marcela Pineda, Nelson Morante, Sandra Salazar, Peter Hyde, Tim Setter, and Hernán Ceballos (2018). Induction of flowering II: night breaks as an alternative for photoperiod extension
3. L. Marcela Pineda, Peter Hyde, Tim Setter, Nelson Morante, Sandra Salazar, and Hernán Ceballos (2018). Induction of flowering III: the potential of plant growth regulators
4. L.M Pineda, B. Yu, T. Yinong, N. Morante, S. Salazar, and H. Ceballos (2018). Induction of flowering IV:the potential of pruning young branches

A plenary presentation was also made during the GCP21 conference:
H. Ceballos (2018). Progress and challenges in our understanding of cassava breeding and genetics. At least two manuscripts are under preparation for their publication in peer-reviewed journals.