INDUCTION OF FLOWERING IN CASSAVA
Agreement No. 67724-10566 (under prime agreement No. OPP1048542)
Report of activities. August 2017

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. . There were clear evidences that interesting developments had been achieved at Guangxi Subtropical Crops Research Institute (GSCRI) in China that could benefit research conducted by NextGen. The International Center for Tropical Agriculture (CIAT) would coordinate the linkage between NextGen initiative and GSCRI and also to carry out new research.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***:** The results of the research activities described above will be integrated in a common protocol for the efficient induction of flowering in Guangxi Province in China and elsewhere and 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 would have been produced 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 (about six month before normal planting time and just before winter). In March 2016 four new varieties were included among the six planted: SC5, SC9, SC205, GR891, GR911 and NZ19.

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 cannot 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

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 were made.

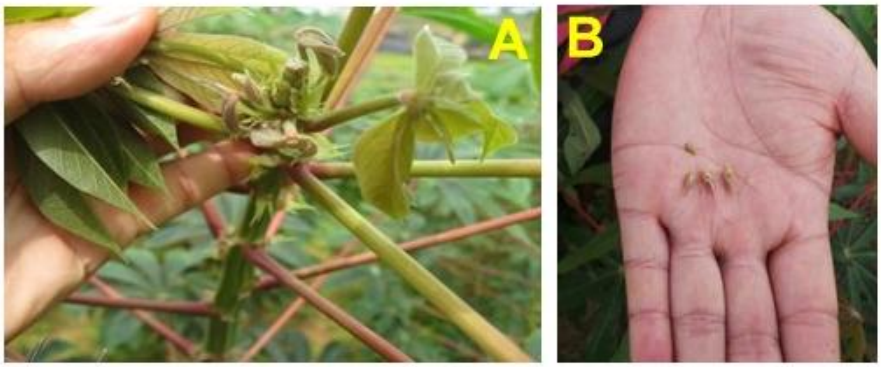

Figure 1. Removal of branches as soon as their presence is detected. **A**. Illustration of a shoot where branches can be identified. **B**. Illustration of removed branches from the apical shoot.

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 the 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 branches that plants eventually produce (Figure 1), the flower racemes remain in the apical extreme of the plant, which can no longer grow (Figure 2). Fruits are harvested before winter time and to start 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. Table 1 presents information in this regard. The earlier the removal of branches the higher the number of female flowers counted across the different genotypes. Number of male flowers remain relatively high. Size of raceme was also largest when reproductive branches were removed as early as March first. However, there are some differences among genotypes. Development of complete flowers (hermaphrodite), is of common occurrence in plants handled following the protocol developed at GSCRI. In this type of flowers self-pollinations are feasible and produce seed that can be used in the process of accelerating inbreeding in cassava.

Table 1. Results on male and female flower production and size of inflorescences of branch removal at different times on ratooned plants. Results are the average across six different genotypes.

Date of branching removal	Branches removed			Branches left untouched		
	♀flower (#)	♂ flower (#)	Raceme length (cm)	♀ flower (#)	♂ flower (#)	Raceme length (cm)
March 1	4.4	32.2	18.2	0.4	7.2	5.6
March 10	4.0	28.4	17.6	0.2	5.4	6.2
March 20	4.2	36.2	16.7	0.4	9.2	6.8
March 30	3.6	21.8	15.8	0.1	3.2	4.2

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 on 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.

Scientists at GSCRI conclude that flowering in cassava is a complex regulatory process within the plant, which is subject to the combined effects of many factors such as light, temperature, rainfall, accumulation of endogenous substances, water and fertilizer management level, etc. Genetic factors are also important in defining flowering, particularly in tropical environments. Due to the impact of climate, cassava seeds in Guangxi can only be obtained when flowering takes place before September each year. Few conclusions can be drawn from our experiences in the past two years.

a. The earlier the planting the better the results. When cassava is planted at the normal dates in February flowering is late and only few fruits, at best, can be obtained. If earlier planting could be done, for example in January then results may be improved. This is why the alternative or ratooning plants that are left permanently in the field has been so successful. The alternative of planting during the fall in the previous year is also useful.
b. The removal of reproductive branches is a second important component of GSCRI protocol inducing earlier flowering. The earlier the removal (e.g. the smaller the developing branches in the shoot) the better. Removing the branches before March 10 resulted in 100% of the plants flowering as early as June 1st.

2.3 Implications for NextGen

Results obtained by GSCRI are valuable for the subtropical environment at high latitudes where they are located (> 22°N) but not so relevant for tropical environments. Key components of the protocol is planting or ratooning plants before winter (e.g. six months earlier than normal) and pruning branches when plants have already initiated flowering. The

system, therefore, requires the first flowering event to take place for implementing the protocol.

During the NextGen meeting at Ibadan in March 2016 it was decided that results obtained by GSCRI are real the protocol would have little relevance for the tropical environments prevailing in Africa. This, combined with progress based on different strategies (described below) lead to the decision to cancel further collaboration with GSCRI.

3. Research conducted at International Center for Tropical Agriculture (CIAT)

3.1 Induction of flowering through grafting

In addition to the advances made by GSCRI prior to the signing of the research agreement CIAT had also been conducting its own research. Namely, for three years CIAT had been working with the grafting techniques originally suggested by Dr. Tim Setter (Cornell University). A research article was published described this technology: **Ceballos H, JJ Jaramillo, S Salazar, LM 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 article describes that six non- or late-flowering genotypes were selected for grafting on a profuse, early flowering understock. Varying number of successful grafts per genotype were obtained. Grafts failed to 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. There was an interesting genotype-dependent response:

- One genotype failed to branch or flower, independently of the origin of the cuttings.
- Five genotypes branched but did not produce flowers. Plants from grafted cuttings tended to branch earlier particularly after the second branching event.
- 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 ordinary (non-grafted) stems.

This is the first report of grafting effects on the induction of flowering in cassava. Results indicated a delayed effect of grafting which was obviously genotype-dependent and could be observed only when cuttings from the grafted stem were planted. Results from this study also demonstrated that there can be branching without apparent production of flowers. This may be a common phenomenon but it has not been observed in day-to-day work making crosses in cassava. The differential reaction among genotypes observed after the grafting experiment may prove to be important in the ongoing research described below.

3.2 Induction of flowering through extension of the photoperiod: the "red light district"

Preliminary research conducted at Cornell University suggests that earlier flowering may be achieved (at least in some genotypes) through manipulation (e.g. lengthening) of the

photoperiod. An experiment was planted at CIAT Experimental Station in Palmira Colombia. Six genotypes were chosen, as follows:

- An "asparagus" clone with sessile leaves (no petiole) and non-branching (**Figure 5**).
- The genotype that did not branch nor flower from the grafting experiment.
- One of the genotypes that branched but did not flower from the grafting experiment.
- The genotype that branched and flowered earlier in the grafting experiment.
- A late branching/flowering commercial clone (CM4919-1)
- An intermediate branching/flowering experimental clone (GM 971-2).

Four different treatments have been implemented to extend photoperiod through the use of LED red light, thus the idea of a "red light district" (RLD) as follows:

a. Five individual LED per plant (**Figure 5**).
b. Two clusters of LED light per plant.
c. One string of LED tape per plant (**Figure 5**).
d. Two strings of LED tape (tentatively for the first and second fully expanded leaf).

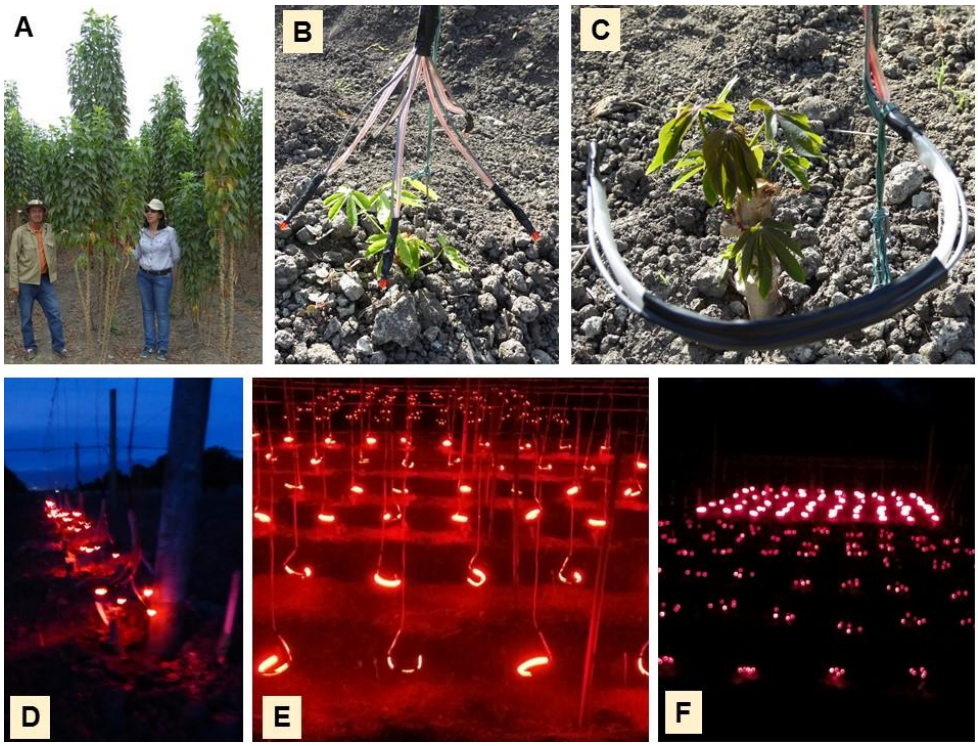

Figure 5. Induction of flowering through extended photoperiod in the RLD. **A**. Illustration of the "asparagus" phenotype; **B**. Illustration of the system with five individual red LEDs per plant; **C**. Illustration of the LED tape used as another treatment; **D**. Photograph taken at sunset of the treatment with five individual LEDs; **E**. Illustration of the LED tape treatment in the foreground and the individual LEDs in the background; and **F**. Illustration of the individual LEDs in the foreground and the LED tape in the background. Notice the higher intensity of light for the tape vs individual LEDs.

Results from the RLD are summarized in Figure 6. 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 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 with extended photoperiod 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 7 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.

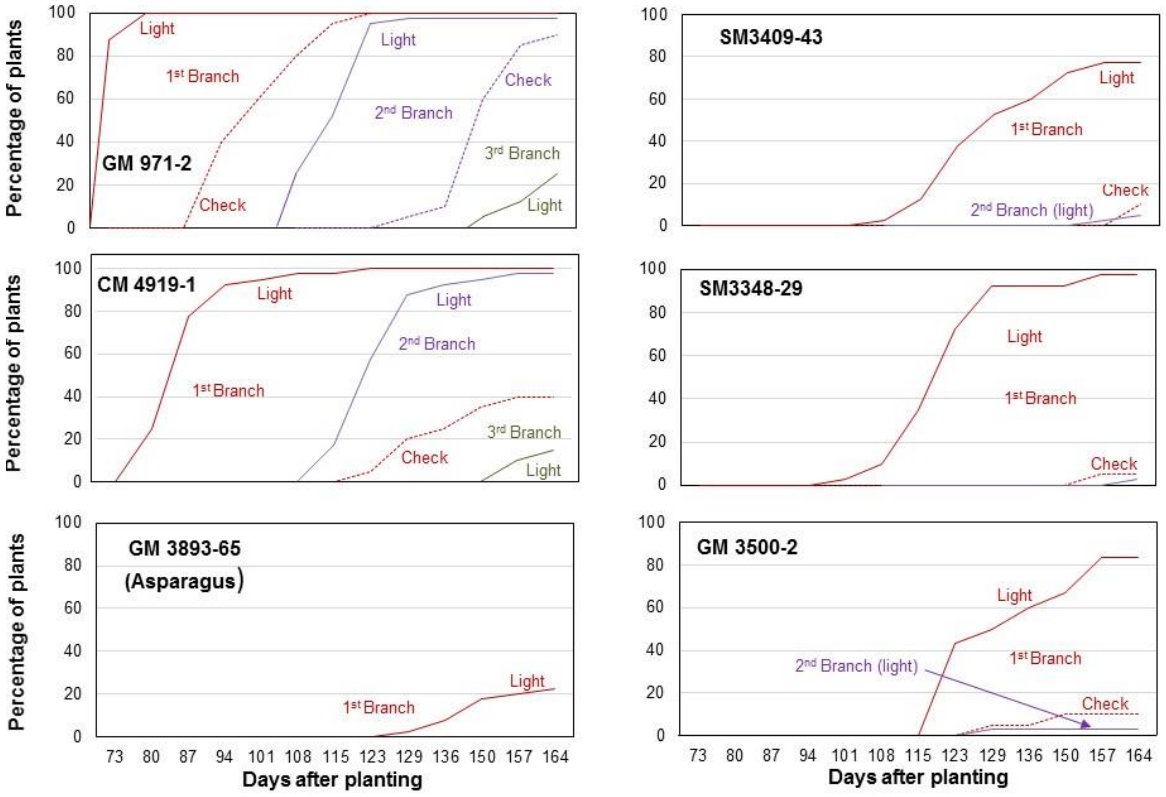

Figure 6. Results for the induction of flowering in the RLD. **GM 971-2** is an early flowering genotype; **CM 4919-1** is a late flowering genotype; GM 3893-66 ("*asparagus*" cassava) does not flower in less than a year; **SM 3409-43** was selected from the grafting experiment (did not branch, nor flowered); **SM 3348-29** branched and flowered in the grafting experiment; **SM 3500-2** branched but not flowered in the grafting experiment.

Figure 7. 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.

3.3 Induction of flowering through application of plant growth regulators (PGR)

Research coordinated at Cornell University and conducted in Nigeria and Uganda suggests that PGR may enhance flowering as well as fruit and seed set. Four different treatments will be used to contribute with a new location for the ongoing work in Africa (all with Tween 20):

- 0.5 mM Silver thiosulfate (STS)
- Benzyladenine - MaxCel (BA)
- STS + BA
- Water control

The same genotypes used in the red light district were also employed for this experiment. Spraying (see Figure 8) 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 9). 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 8. Illustration of the methodology employed in the application of the plant growth regulators.

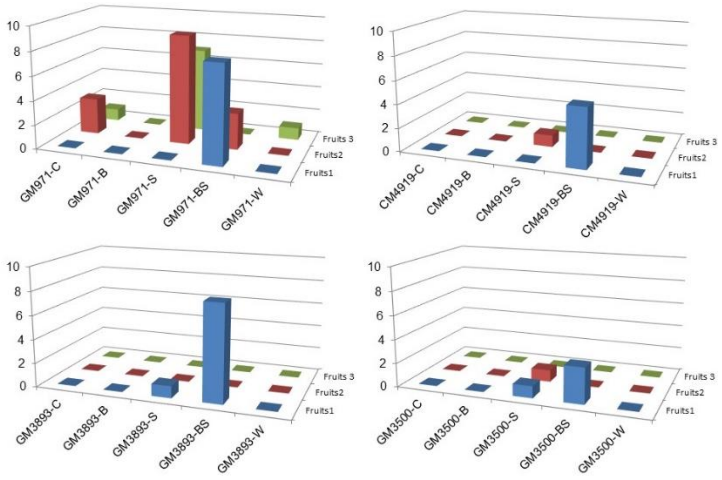

Figure 9. Response of four genotypes to the application of plant growth regulators (number of fruits in the three levels of branching (Fruits 1, 2 and 3, respectively). The name of each genotype is followed by a code standing for the check (-C); application of benzyladenine (-B); silver thiosulfate (-S), both regulators (-BS), and water+ tween 20 (-W).

3.4 Concluding remarks

- There were remarkable, genotype-dependent responses to the RLD and PGR.
- Harvest of fruits in the RDL could be made 4-6 months earlier.
- PGR allowed harvest of fruits in the "asparagus" genotype about 9 months earlier.
- There were no differences in the response to different light intensities.
- A low intensity of red light (0.5 to 1.0 lux) is enough to elicit a response in the plant.
- The earlier the stimulus are initiated the better.
- Some genotypes are still recalcitrant to flower.

5. Ongoing research

In July 2017 CIAT initiated the planting of a new set of experiments related to flowering induction that takes advantage of the results obtained the previous season. The experiment was planted on July 21st. The objectives of the new set of experiments are as follows:

1. Validate the effect of extended photoperiod (RLD) and plant growth regulators (PGR) in the six genotypes used the previous season. Doing this will provide results for a second season which will lead to a publication.
2. Combine photoperiod extension (all night long) with PGR in the six genotypes used the previous season. Each combination of stimuli is represented by 10 plants. In five of these plants PGR will began 75 DAP (as in the previous season) and in the remaining five plants application of PGR will start 45 DAP. The objective of this study is to assess the potential synergies among the two type of stimuli (RLD + PGR), which could hopefully widen the range of responsive genotypes and/or further reduce the time required for flowering.
3. Assessment of the effect of "*night breaks*" as an alternative for all-night illumination. Ideally the implementation of a RLD in crossing blocks will be based on the use of solar panels so it does not require access to "street electricity". Night breaks would drastically reduce the amount of energy required to elicit flowering which in turn should reduce to cost of a system based on solar panels (e.g. reduce the cost for batteries). Breaks will take place around mid-nigh and lights will be on for 30, 60, 90, 120, 180 and 240 minutes.
4. Assessment of different lighting alternatives. Also aiming at implementing an efficient set up for a RDL that can be implemented in "commercial crossing blocks" of cassava different sources of light will be evaluated. Red LED tapes (5m long) will be assessed on top of single rows, or covering up to five rows. Also a 50 watts red light led reflector in a large area planted with cassava (120 m^2) will be evaluated (Figure 10).
5. Plants from few genotypes will be grown and apical meristems collected for histological analysis. CIAT would like to determine how early inflorescence primordia can be observed in the apical meristem in relation to time branching can be visualized. The timing of initiation of inflorescence development is important for identifying changes in gene expression and hormonal status of the plant.

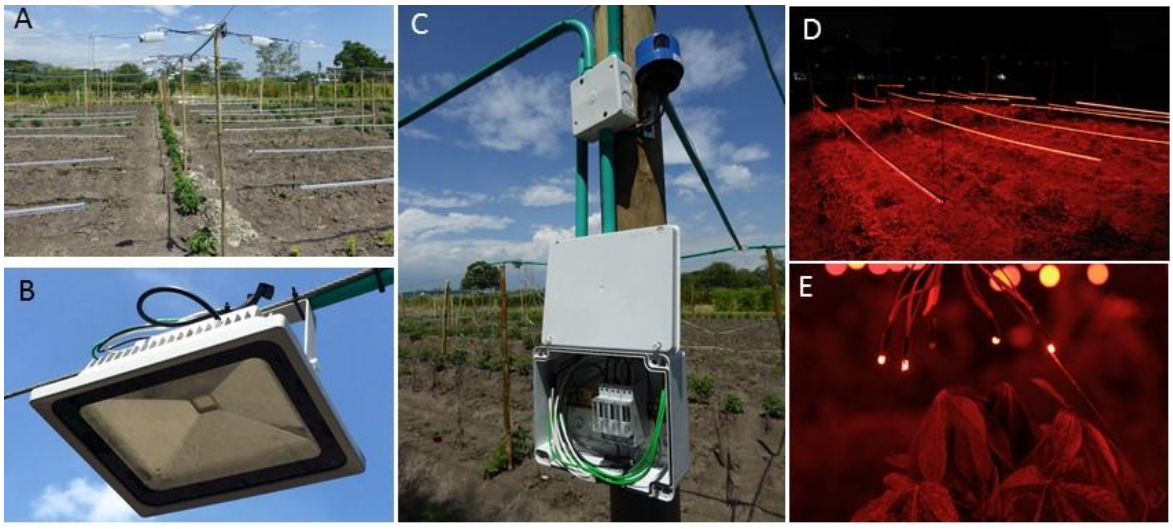

Figure 10. Illustration of new sources of red light for the RLD. **A**: Example of 5m long LED tapes; **B:** A 50 watt red light reflector; **C**: Control box for three of the six night break treatments; **D**: Effect of LED tapes; **E**: Effect of five individual LEDs (≈ 0.5 1.0 lux), which is enough to elicit a response in the plant.