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Induction of flowering in cassava (*Manihot esculenta* Crantz) using plant growth regulators, pruning and extended photoperiod through night-breaks

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Cassava is a food security crop in Kenya, whose production is hampered by cassava brown streak disease (CBSD) and cassava mosaic disease (CMD). These challenges require urgent resistance breeding. Crossing of varieties in cassava is disadvantaged by poor and asynchronous flowering. This study aimed to induce and enhance flowering in CBSD immune and resistant clones with variable flowering characteristics. Two experiments were established at Kenya Agricultural and Livestock Research Organization, Thika. The First experiment evaluated the effects of plant growth regulators and pruning on flowering, laid in a randomized complete block. The Second experiment evaluated the effects of night breaks on flowering, set in RCBD with twelve replicates. In the first experiment, treatment resulted in significant ($p < 0.001$) increased number of female flowers, fruits and seeds. However, the treatment did not reduce the number of days to flowering and height to first branching. In the second experiment, treatment resulted in significant ($p < 0.001$) increased number of female flowers and fruits, reduced days to flowering, height to first branching, and number of nodes to first branching. Approaches tested here can be readily deployed in enhancing flowering and accelerating cassava breeding thus contributing to improved food security.

Key words: Plant growth regulators, pruning, night breaks, flowering, cassava.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz.), of the Euphorbiaceae family, is a tropical plant cultivated as an annual crop for its starchy storage roots that are consumed by approximately half a billion people in Africa daily. It is also an important staple in Latin America and the Caribbean (Alene et al., 2018). The leaves, which are

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consumed in some African countries as a vegetable, are a rich source of vitamins, minerals, and proteins (Dodo, 2020). Cassava is increasingly being utilized as an industrial raw material for manufacturing pharmaceuticals, industrial starch, biofuels and other products (Githunguri et al., 2017). In Kenya, cassava is the second most important root crop after potato and is grown widely in western, coastal and eastern regions of the country (Githunguri et al., 2017). The increasing effects of climate change on food security and income generation have shifted attention to this resilient staple starch crop.

The production of cassava is challenged by various biotic and abiotic stresses with pests and diseases estimated to be second to drought in affecting cassava productivity (Jarvis et al., 2012). The most devastating insect pests that affect cassava are thrips, whiteflies, and mites (Milenković et al., 2020, Lebot, 2020). Cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* is the most economically important bacterial disease that affects the crop (Fanou et al., 2018). Cassava is also vulnerable to at least 20 different viruses, of which those causing cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the most economically devastating resulting in annual production losses of more than US$ 1 billion annually (IITA, 2014). CBSD, caused by cassava brown streak virus and Ugandan cassava brown streak virus, has existed in East Africa since the 1930s when it was first reported in coastal Tanzania (Storey, 1936). In 2004, CBSD was reported in Uganda and began to spread fast in the surrounding area, causing substantial losses to useable production. The urgent need to improve yields, agronomic traits, and consumer preferences, as well as address the calorific demands of the increasing population, necessitates efficiency in the genetic improvement of cassava through national and international breeding programs. The primary objective is to combine and accumulate favorable alleles into superior genotypes by crossing donor parents. A crucial factor in achieving this progress is the number of crosses, or recombination events that can be made. However, in cassava, this is constrained by several factors, including poor flowering, non-synchronized flowering of prospective parents, pollen viability issues, genotype incompatibility, and low seed set (Ibrahim et al., 2020).

Cassava is monoecious with female and male flowers produced separately within the same plant. Female flowers occupy the lower part of the inflorescence while the male flowers, with multiple reduced stamens, occupy the upper part.

Despite the female flowers opening 10 to 14 days before the male flowers, the stigma is only receptive to pollen for a maximum of three days after opening (Ceballos et al., 2017). Female flowers have a trilocular ovary, meaning that successful pollination results in fruits capable of producing three seeds (Perera et al., 2013).

Initiation of flowering in cassava occurs at the apical meristem and is always associated with forking or branching, creating a base where the flower structure and/or inflorescence develops and results in a series of tiers (Hyde et al., 2020). Most cassava genotypes produce non-viable flowers at first forking due to immature abortion (Adeyemo et al., 2019). Some branching cassava types may fork four or more times, creating four or more tiers, whereas erect plant types may not flower and branch at all. The later genotypes have limited use as breeding material irrespective of whether they carry important breed able traits (Baguma et al., 2023). The erect types are however preferred by farmers owing to their amenability to some agronomic practices and mechanization as well as ease of obtaining planting materials (Baguma et al., 2023).

Crossing between genotypes requires synchronized flowering, a challenging undertaking due to the differences in flowering of the breeding lines. Some genotypes flower early at four or five months after planting (MAP) whereas others flower up to 10 MAP (Ceballos et al., 2017). Some varieties do not flower at all in most environments and cannot be used in breeding.

Attempts to enhance, accelerate and synchronize flowering in cassava for crop improvement have used approaches such as plant growth regulators (Hyde et al., 2020), grafting (Ceballos et al., 2017), pruning (Pineda et al., 2021), genome editing (Bull et al., 2017) and extension of photoperiod (Baguma et al., 2023, Santos et al., 2023). Plant growth regulators (PGRs) are biochemicals synthesized by plants in small quantities often in response to external stimuli. They can bring about rapid changes to the growth morphology and physiology of the plant from seed germination to senescence (Amanullah et al., 2010). They can provoke reactions in plants depending on the targeted plant's tissues, developmental stage, hormone concentration, uptake and storage of water and other nutrients, and climatic conditions (Ferguson and Grafton-Cardwell, 2014). The use of plant growth regulators has successfully been utilized to induce flowering in both angiosperms and gymnosperms (Aliyu et al., 2011). Efforts to regulate and influence flowering in cassava using PGR have been made, with the ultimate goal of increasing the number of female flowers (Baguma et al., 2023; Santos et al., 2023; Ceballos et al., 2017; Pineda et al., 2021; Hyde et al., 2020).

The PGR candidates that have been evaluated on cassava under greenhouse conditions include cytokinins, auxins, gibberellins, anti-GA, jasmonic acid, and salicylic acid, with anti-ethylene PGR and silver thiosulfate (STS) showing promising results (Hyde et al., 2020). The anti-ethylene growth regulator, STS, has been shown to improve inflorescence and flower development as well as flower longevity which results in increased female flower numbers (Hyde et al., 2020). Benzyl adenine (BA) is a cytokinin which is another PGR group utilized in cassava, which plays a role in flower sex determination by

feminization of plants. It acts by modifying the apical meristem of male flowers leading to the formation of gynoecium and pistil (Luo et al., 2020).

Ceballos et al. (2017) and Souza et al. (2018) successfully induced flowering through grafting, a technique that leverages the mobility of the flowering signal (Notaguchi et al., 2009). This grafting technique yields valuable clones that retain the preferred traits of the maternal genotype (Milenković et al., 2020). Pruning by removing the young developing branches, just below a newly initiated inflorescence, has been shown to improve flower development in cassava and increase the total number of flowers, fruits, and seeds (Pineda et al., 2021). This method enhances transport of energy, increases light and air uptake and provides maintenance and support of the photosynthetic process (Harkulkar et al., 2022).

Extended photoperiod with red light emitting diodes of 625 to 635 nm has been utilized in inducing flowering which resulted in increasing numbers of female flowers and reduced time to flowering (Pineda et al., 2021). Long days have been used to induce flowering in cassava and are created by extending the photoperiod using artificial lighting or interrupting the night (Harshitha et al., 2021).

Genome editing tools have been utilized in enhancing cassava flowering by adding the Arabidopsis FLOWERING LOCUS T gene into the genome-editing cassette (Tyagi et al., 2021). CRISPR/Cas9-mediated disruption of Multiple TFL-like floral repressors has been utilized in hastening flowering in cassava (Odipio et al., 2017).

Although methodologies exist to induce and enhance flowering in cassava globally, they have not been tested in Kenya or in cassava genotypes with immunity and/or resistance to CBSD that exhibit poor flowering characteristics. It is crucial to test flowering induction techniques in areas where they are most needed and under natural conditions where flowering interventions are necessary.

Kenya, where the current studies were conducted, is located near the equator, with relatively consistent day and night lengths throughout the year. As an important cassava-growing and consuming country, Kenya faces significant challenges due to CBSD's negative impact on production. For these technologies to be practically useful, they must be readily available locally and affordable.

The studies conducted here aimed to induce flowering by extending the photoperiod through night breaks using white light, plant growth regulators, and pruning in eight cassava clones from South America that are immune or highly resistant to CBSD. Although red light has been used to extend the photoperiod (Santos et al., 2023; Baguma et al., 2023), white light is more readily available in Kenya. Furthermore, using night breaks instead of extending the light period consumes less electricity, making it a more cost-effective approach. The knowledge and experience gained from this study provide a strong foundation for determining the number of crosses that can be made in one season, facilitating speed breeding for CBSD resistance and other desirable traits.

MATERIALS AND METHODS

Location

The experiments were conducted at Kenya Agricultural and Livestock Research Organization, Horticulture Research Institute, Thika, Kenya. The station lies at an altitude of 1548 m above sea level (masl) latitude 00°59' S, and a longitude 37°04'E. The mean temperatures and rainfall experienced over the period of the experiment was 21 to 28°C and 1200 to 1450 mm, respectively. The soils are well-drained, deep dark red and friable NITOSOLS (Jaetzold and Schimdt, 2009).The location is a low pressure area for CMD and CBSD (Peninah et al., 2022), hence it was selected for this experiments as the clones used are susceptible to CMD and immune or high resistant to CBSD.

Germplasm and experimental design

Eight clones with immunity or high resistance to CBSD were obtained from the International Institute of Tropical Agriculture (IITA) and were chosen based on variability in time to flowering and resistance and/or immunity to CBSD (Sheat et al., 2019) (Table 1). The immune clones were DSC 120 (COL144), DSC 196 (ECU 41), DSC 269 (PER 556) and DSC 258 (PER 333), and highly resistant clones were DSC 272 (PER597), DSC 257 (PER 315), DSC 251 (PER 226) and DSC 248 (PER 206) (Sheat et al., 2019).

Experiment 1: Induction of flowering using plant growth regulators and pruning

The experiment, consisting of treatments and controls, was set up in a Randomized Complete Block Design (RCBD) with four replications during the 2021/2022 growing season. The germplasm used for this experiment is described in Table 1. Plants were spaced 1m by 1m, with each plot containing five plants. Controls without any treatment were included for each genotype per replication.

The plant growth regulators used were BA and STS. STS was prepared by mixing 1 part 0.1 M silver nitrate ($AgNO_3$) with four parts 0.1 M sodium thiosulfate (Na_2S2O_3), whereas BA solution was prepared by diluting a 1.9% (w/v) BA with distilled water to respective concentrations as described by Oluwasanya et al. (2021).

The PGR treatments were initiated when most plants had reached a height of 45 to 60 cm. The BA regulator was applied by spraying 5 ml of 0.5 mm to the shoot apex every seven days, targeting 3 to 5 young immature leaves. STS was applied every 14 days using the petiole method, where the leaf lobe was removed using a surgical blade, the petiole immediately rinsed in distilled water and inserted into a 15-ml conical-bottom centrifuge tube containing 2.5 ml STS solution and secured onto the plant (Figure 1).

It was expected that STS would be taken up via the petiole into the xylem, from which it would be distributed internally up to the apex. Petioles were allowed to remain immersed in STS solution for 72 h, after which the tubes were removed.

Table 1. List of the clones used in this study and their characteristics.

Clone	Name	Flowering status at KALRO Thika	S tatus of CBSD resistance	Experiment used*
DSC 120	COL144	Medium flowering	Immune	Experiment 1 and 2
DSC 196	ECU 41	No flowering	Immune	Experiment 2
DSC 248	PER 206	Late flowering, high abortion rate	Resistant	Experiment 1
DSC 251	PER 226	Early flowering	Resistant	Experiment 1
DSC 257	PER 315	Medium flowering, high abortion rate	Resistant	Experiment 1 and 2
DSC 258	PER 333	No flowering to late flowering, high abortion	Immune	Experiment 1 and 2
DSC 269	PER 556	No flowering, to late flowering, high abortion	Immune	Experiment 2
DSC 272	PER597	Medium flowering, High abortion rate	Resistant	Experiment 2

*Experiment 1: PGR and pruning; Experiment 2: Extension of photoperiodism.
Source: Sheat et al. (2019).

A B C

Figure 1. Approaches used in the induction of flowering using plant growth regulators and pruning. A-Spray method, B-Petiole method and C- inflorescence after pruning. Red arrows show pruned branches at the second fork.

Shoot meristems were inspected three times a week using a hand lens (10× magnification) to identify forking and flowering. In plants that had started forking, young branches, more or less 2 cm, were removed carefully using forceps so as not to damage the inflorescence (Pineda et al., 2021, Oluwasanya et al., 2021). Pruning was done to plants treated with PGR and on the second forking event/ tier (Figure 1).

Experiment 2: Induction of flowering using extended photoperiod by night break

Experiment 2, designed to determine the effect of extended photoperiod by night break on the time to flowering, was set up in a RCBD with 12 replicates. The germplasm used consisted of six cassava clones: DSC 120, DSC 196, DSC 257, DSC 258, DSC 269, and DSC 272 (Table 1). A control experiment without supplemental lighting was conducted one kilometer away, also in an RCBD. Each plot contained three plants, spaced 1 x 1 m, covering a total field area of 12 by 18 m (Figure 2).

Source of light

The lighting structure measured 18 × 12 × 10 m (Length × Width × Height) and had six, 100-W white light-emitting diodes (LED) distributed across the set-up (Figure 2). A LI-180 spectrometer (Licor, USA) was used to measure Photosynthetic Active Radiation (PAR) using the photosynthetic photon flux density (PPFD) module. An automatic timer turned the lights on at 10:30 pm and off at 1:30 am allowing for a three-hour night break. The night breaks were affected from planting until harvesting at 10 months after planting (MAP).

Field management, data collection and analysis

Field management for the two experiments followed the standard procedures for cassava. Manual weeding was done in and around all the plots. Irrigation was provided via hand watering in and around the plots uniformly. Data on induction of flowering using PGR and pruning was collected per plant up to second tier of branching only. Weekly visits and data collection allowed for the identification of flowering and branching. Data on the following variables was collected for the first experiment: days to first flowering; plant height to first branching; number of female flowers, and total number of fruits and seeds. On the second experiment, data on the following variables was collected; number of female flowers, number of fruits, Number of nodes to first branching, plant height to first branching, days to 1st flowering, shoot weight and

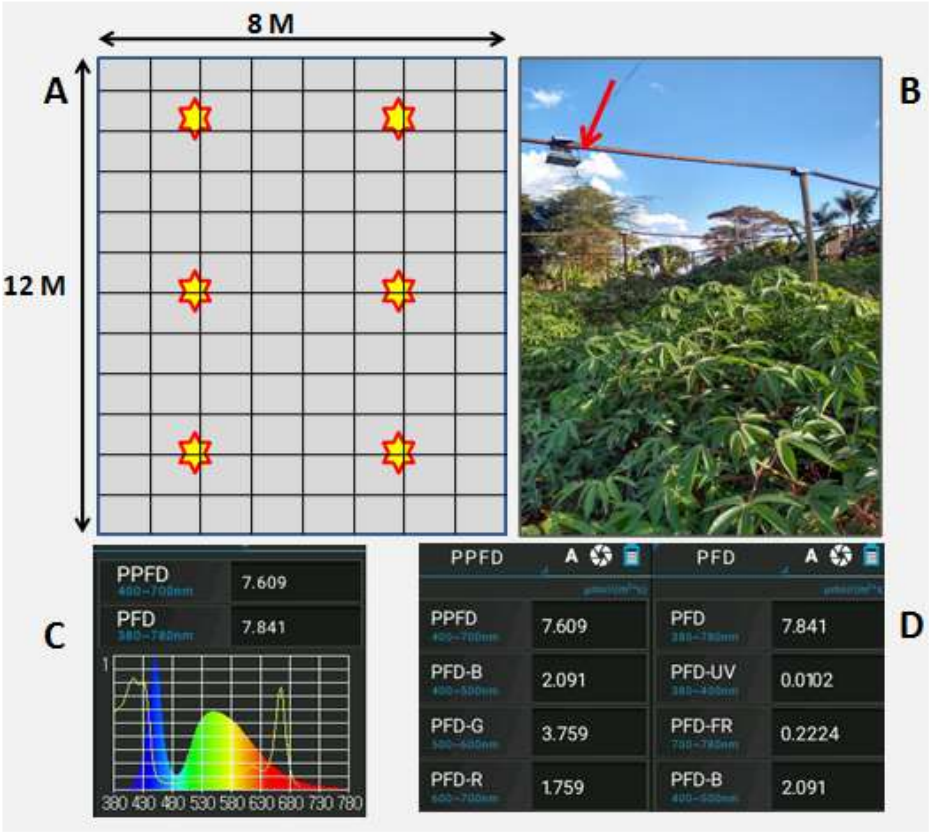

Figure 2. Light experiment set-up and sample light levels. A - Schematic representation of the light structure, B - Cassava plants under light set-up, C - A sample quantification of Photosynthetically Active Radiation during night break showing total PAR and D - quality of PAR under PPFD and photosynthetic flux density (PFD). Red arrow indicates LED light position.

root weight. Analysis of variance and Pearson correlation was performed using SAS 9.4 statistical software.

RESULTS

Induction of flowering using plant growth regulators and pruning

A combination of PGRs (BA and STS) and pruning were used to induce flowering in five CBSD-resistant and immune cassava clones. The use of PGRs as well as pruning resulted in a higher number of female flowers, number of fruits, and number of seeds relative to the control. The average number of female flowers differed between treatments and controls, where treatment in all clones outperformed the non-treated controls (Figure 3). The mean numbers of female flowers in the treatments were 21.9, 22.9, 18.45 and 11.9 for the clones DSC 248, DSC 251, DSC 257 and DSC 258 respectively. The non- 4.55 respectively (Figure 3, Supplementary Table 1). 251, DSC 257 and DSC 258 were 8.75, 7.9, 8.95 and treatment controls for the same clones DSC 248, DSC

Higher abortion levels of flowers were observed in the control experiment, especially on DSC248, DSC 257 and DSC 258 relative to the treatment. The average number of fruits in all the treatment clones was higher compared to the controls (Figure 3, Supplementary Table 1). The average numbers of fruits in the treatment were 8.3, 10.35, 5.3, 10.05 and 1.4, 3.65, 1.4 and 1.75 in the control for the clones DSC 248, DSC 251, DSC 257 and DSC 258 respectively. Similarly, the average number of seeds in all the treatment plants was higher compared to the controls (Figure 3, Supplementary Table 1). The average plant height to first branching varied among clones, but the effect of treatment was not statistically significant relative to the control (Table 2). Although the mean days to flowering differed among clones, there was no significant variation in the treatments and controls (Table 2). The clone DSC 120 was excluded from the analysis as it only flowered in the third tier, whereas only clones that flowered at the second tier were considered for analysis. Despite the application of PGR and pruning, the clone unfortunately aborted the inflorescence at the second tier.

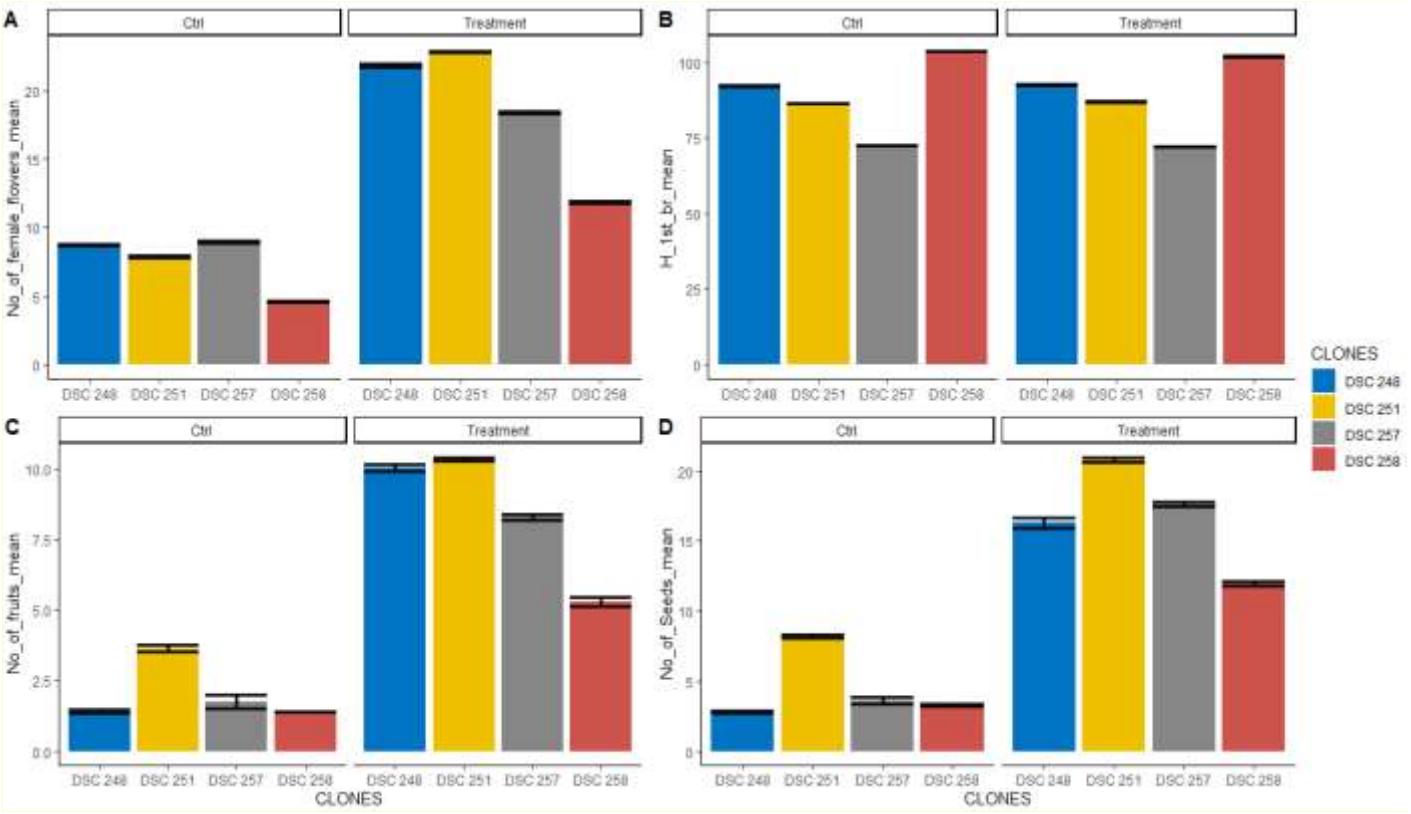

Figure 3. Bar plots of means under PGR and pruning treatment for the flowering parameters: A-Mean number of female flowers under pruning and PGR treatment (Treatment) and no pruning and PGR control (Ctrl), B-Mean number of fruits under pruning and PGR treatment (Treatment) and pruning and PGR control (Ctrl), C-Mean height to first branching under pruning and PGR treatment (Treatment) and no pruning and PGR control (Ctrl) and D-Mean number of seeds under pruning and PGR treatment (Treatment) and no pruning and PGR control (Ctrl).

All the main sources of variation (clones, treatment, and the clone*treatment interaction) were observed to have significant effects ($p < 0.001$) on number of female flowers and number of fruits. Plant height to 1st branching at ($p < 0.001$) was observed to vary significantly across the different clones; however, clones and treatment interactions had no effects on plant height (Table 2). Clones and treatment as sources of variation had an effect on days to flowering ($p < 0.001$), whereas clones and treatment interaction was not significant. Clones and treatment as sources of variation had effects on the number of seeds ($p < 0.001$), whereas clones by treatment interaction had an effect on the number of seed ($p < 0.01$) (Table 2).

Pearson correlation among flowering parameters under PGR and pruning treatments

There was significant **(**$p < 0.001$) positive correlation of $R^2 = 0.96641$ between number of female flowers and number of fruits (Table 3), similarly there was a strong positive correlation of $R^2 = 0.92302$ between number of female flowers and number of seeds ($p < 0.001$) (Table 3). The number of fruits correlated positively with the number of seeds at $R^2 = 0.97433$ **(**$p < 0.001$) (Table 3).

Days to flowering correlated positively with height to first branching at $R^2 = 0.66057$ **(**$p < 0.001$) (Table 3).

Induction of flowering by extending photoperiod through night break

Apart from the clone DSC 272, flowering was enhanced in all the five clones under the night break experiment relative to the dark night control. The enhanced flowering was observed through an increased number of female flowers where the DSC 120, DSC 196, DSC 257, DSC 258 and DSC 269 had a mean of 155.083, 6.389, 28.083, 1.5 and 0.972 female flowers in the night break experiment and 5.972, 0, 7.056, 0 and 0 in the control (Figure 4 and Supplementary Table 2).

Surprisingly, the clone DSC 272 in the control had a greater number of flowers (average 10.9 than in the night break experiment average 5.6). Despite the enhanced flowering of the five clones in the night break experiment, abortion of flowers was observed in all clones. Abortion was observed in the dark night experiment in all clones

Table 2. Variance analysis of effects of treatment (PGR and pruning) on flowering parameters in four clones.

Source of variation	df	Number of female flowers	Number of fruits	Plant height to 1st branching	Days to flowering	Number of seeds
Replicates	3	0.30	0.63	1.20	56.66	3.20
Clone	3	91.60***	18.52***	1310.38***	36468.06***	67.49***
Treatment	1	1012.50***	332.82***	0.98	1205.41***	1190.72***
Clone × Treatment	3	23.96***	7.61***	2.26	145.88	11.56**
Error	21	0.48	4.79	4.17	54.52	1.79
R^2		0.99	0.99	0.98	0.99	0.97
CV (%)		5.27	9.05	2.31	5.27	12.67

,* =significant at p<0.01;p<0.001, df =degree of freedom, CV (%) =coefficient of variation, R^2=coefficient of determination.

Table 3. Pearson correlation coefficient of flowering parameters of 4 cassava clones under PGRs and pruning.

Parameter of flowering	No. of female flowers	No. of fruits	Plant height to 1st branching	Days to flowering	No. of seeds
No of female flowers					
No of fruits	0.97***				
Plant height to 1st branching	-0.27	-0.17			
Days to flowering	-0.39	-0.43	0.66***		
No of seeds	0.92***	0.97***	-0.19	-0.45	

Coefficient of determination- R^2 ; ***Statistically significant at p<0.001, Number of female flowers, Number of fruits, Days to flowering and Number of seeds.

and particularly on DSC 258 and DSC 269. However, the relative rates of abortion across clones and treatment were not quantified in this study.

Similar to the observations with female flowers, the numbers of fruits were higher in the night break experiment relative to the control (Figure 4 and Supplementary Table 2). There were observable differences in the average number of fruits between clones and across treatment with the clones DSC 120, DSC 196, DSC 257, DSC 258, DSC 269 and DSC 272 having an average of 16.222, 3.444, 5.472, 0.361, 0.222 and 2.472 female fruits under night break and 2.111, 0, 2.556, 0, 0 and 4.833 in the dark night control respectively.

Branching was observed in all the clones under the night break experiment relative to the dark night control experiment where the clone DSC 196 did not branch (Figure 5). Branching under night experiment occurred at a shorter height under night break compared to the control for the clones that branched. The clones: DSC 120, DSC 196, DSC 257, DSC 258, DSC 269 and DSC 272 branched at 63.194, 62.306, 59.833, 57.611, 68.389, and 68.917 cm under night break treatment respectively. In the control the clones: DSC 120, DSC 257, DSC 258, DSC 269 and DSC 272 branched at 92.222, 86.806, 80.889, 76.056 and 92.861, respectively. No branching was observed in the clone DSC 196 in the dark night control. This meant that first branching under night break occurred when the plants were shorter as compared to the dark night control. Similarly, under night break, the number of nodes to first branching was fewer relative to the control (Figure 5 and Supplementary Table 2). The clones DSC 120, DSC 196, DSC 257, DSC 258, DSC 269 and DSC 272 had 15.222, 26.278, 15.667, 15.972, 16.444 and 18.139 under night break treatment. In the control, the clone DSC 196 did not branch while the clones DSC 120, DSC 257, DSC 258, DSC 269 and DSC 272 had 30.5, 23.306, 23.167, 21.722 and 26.583 branches respectively (Figure 5 and supplementary Table 2).

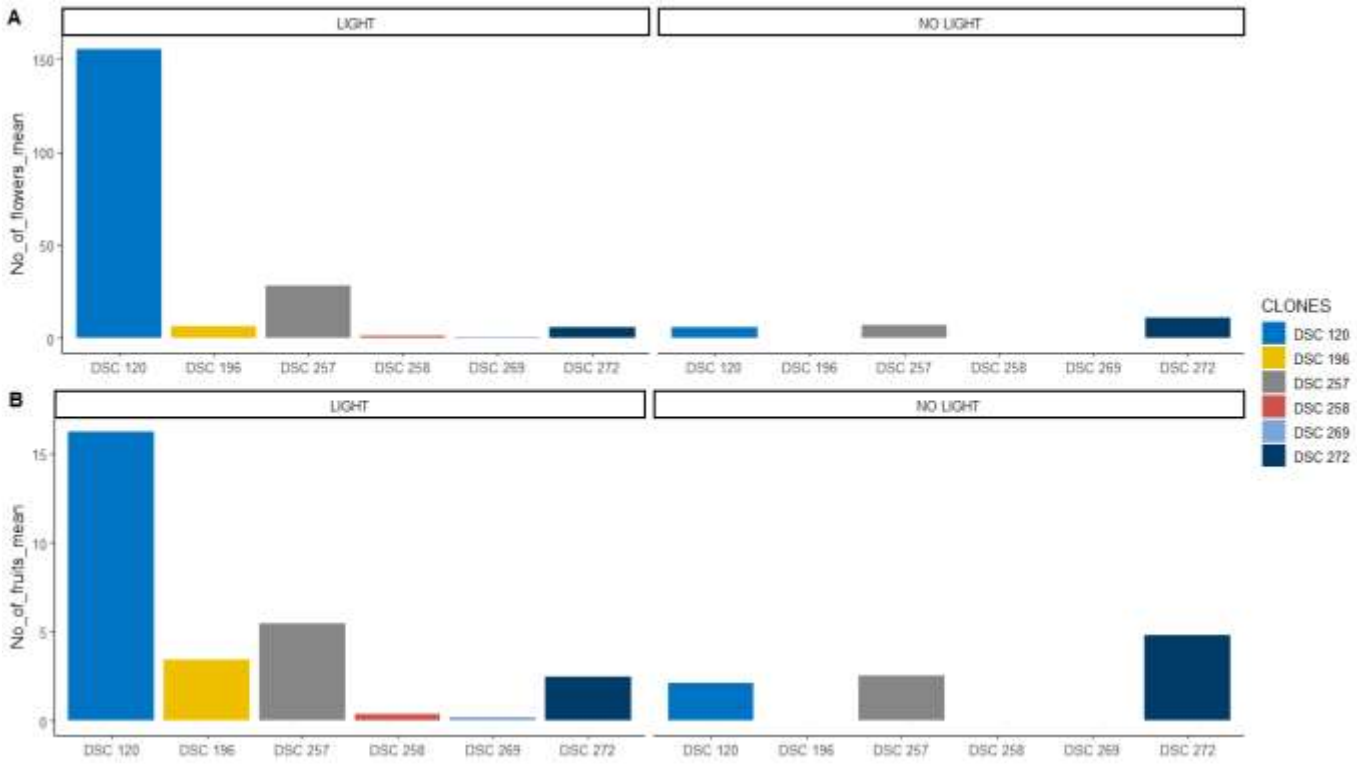

Figure 4. Average number of female flowers and fruits under night break treatment. A- Average number of female flowers under light and no light experiment in six cassava clones; B- Average number of fruits under light and no light in six cassava clones.

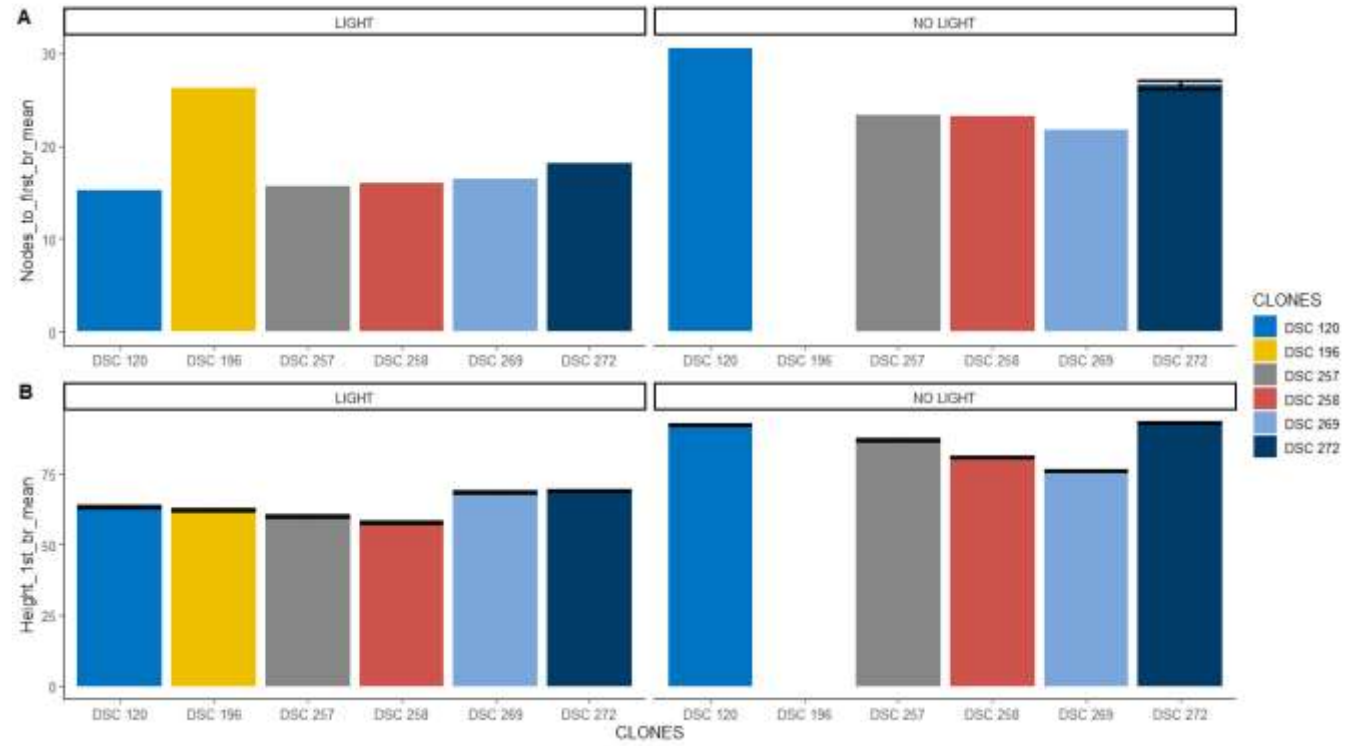

Figure 5. The average number of nodes and height to first branching: A-mean number of nodes to first branching under night break treatment (LIGHT) and dark night control (NO LIGHT); B- mean height to first branching under night break treatment (LIGHT) and dark night control (NO LIGHT).

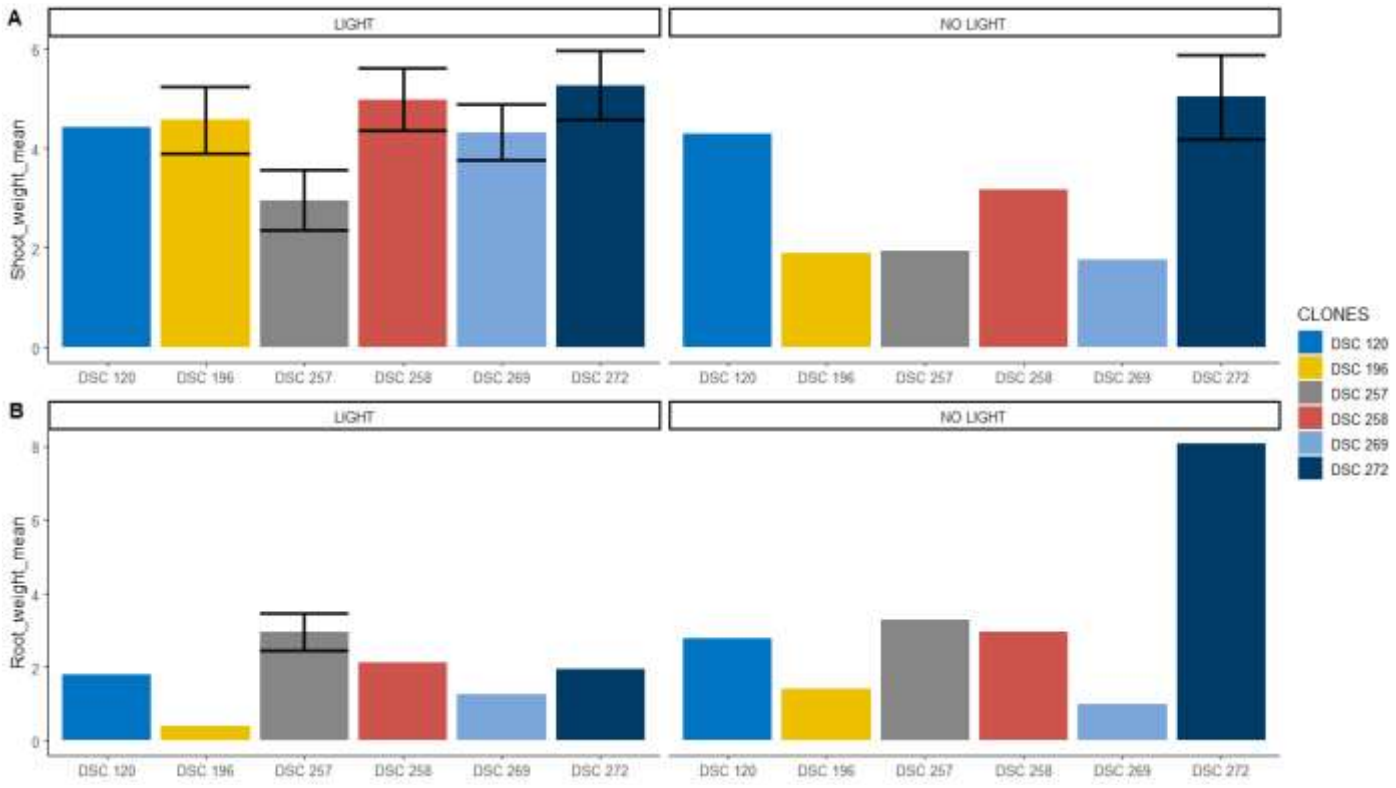

Figure 6. Bar plots of mean wet shoot and root weight under extended photoperiod: A- Average wet shoot weight under night break (LIGHT) and dark night control (NO LIGHT), B - Average wet root weight under night break (LIGHT) and dark night control (NO LIGHT).

Under extended photoperiod, the average shoot weight in five of the six clones was observed to be higher than in the control. The wet shoot weight in the clones DSC 120, DSC 196, DSC 257, DSC 258 and DSC 272 was 2.791, 1.417, 3.284, 2.966 and 8.074 kg respectively. The wet shoot weight in the clone DSC 269 was slightly lower at 0.999 kg compared to 1.262 kg under dark night control experiment. The clones DSC 120, DSC 196, DSC 257, DSC 258 and DSC 272 under dark night control had shoot weights of 1.810, 0.410, 2.962, 2.111 and 1.937 (Figure 6 and Supplementary Table 2).

Extension of photoperiod by night break reduced the days to first flowering (DTF) relative to the dark night control across in all clones. Under the night break treatment, the average DTFs for the clones DSC 120, DSC 196, DSC 257, DSC 258, DSC 269 and DSC 272 were 107.58, 92.92, 87.42, 102.92, 151.75 and 92.33 whereas the DFFs for the same clones under dark night control were 134.08, NA, 106.67, 208.08, 250.08, 133.083 respectively (Figure 7). The clone DSC 196 under dark night control did not flower at all. Clones DSC 258 and DSC 269 flowered but all the flowers aborted. This meant that the clones at the night break flowered early as compared to the clones in the control.

To further confirm if the variations observed in some of the agronomic traits under extended photoperiod were statistically significant, analysis of variance was done using SAS statistical software. All sources of variation; condition (light and no light), clones and clones condition interaction had effects on flowering parameters (Table 4). Condition, clones and condition and clones' interactions had significant effect $p< 0.001$ on number of female flowers, number of fruits, and number of nodes, shoot weight, root weight, plant height to first branching and days to first flowering.

Pearson correlation among flowering parameters in Experiment 2

There was significant ($p < 0.001$) positive correlation between number of female flowers and number of fruits (Table 5). The number of nodes to first branching correlated positively with the plant height to first branching ($p<0.001$) (Table 5).

DISCUSSION

Plant growth regulators and pruning in induction of flowering

The combination of the plant growth regulators BA and anti-ethylene STS, along with pruning, successfully induced and increased the number of female flowers,

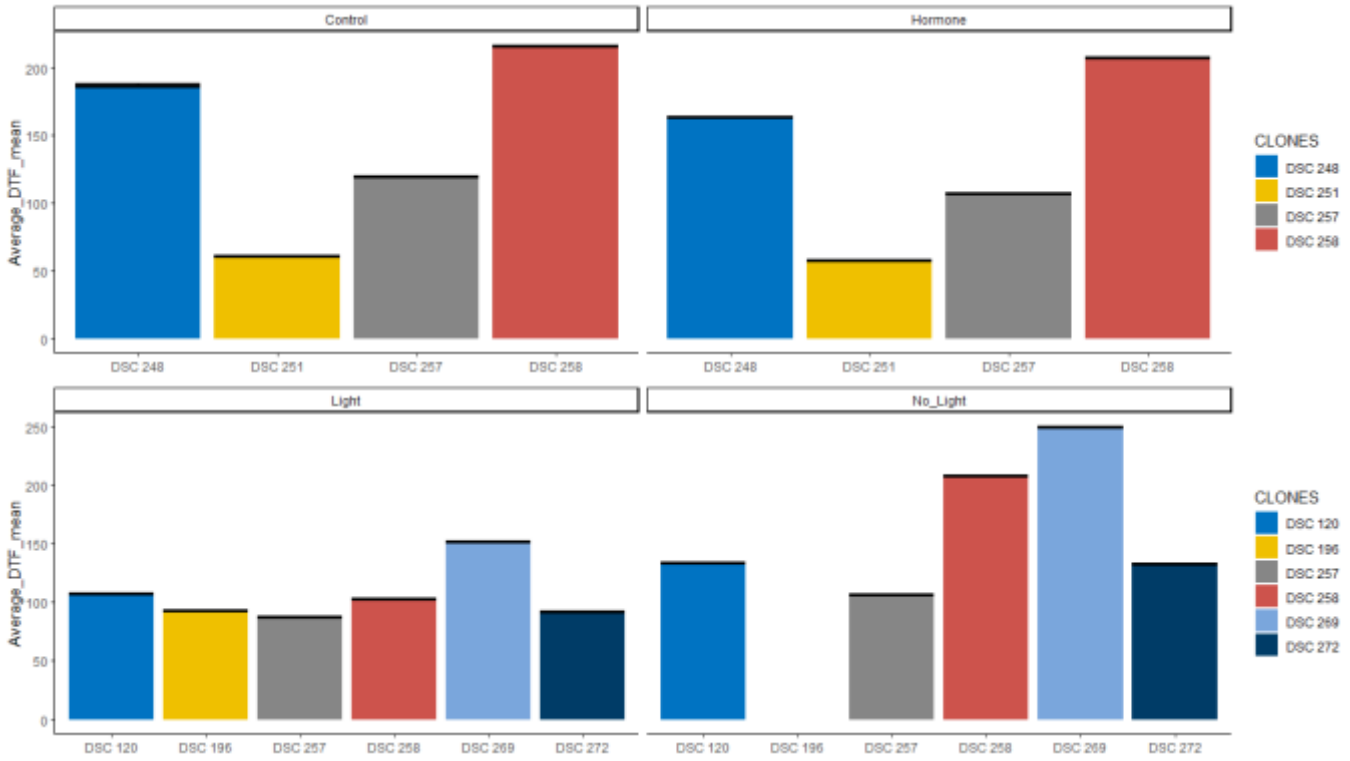

Figure 7. Bar plot of mean days to first flowering under night break and dark night control: A-Mean number of DTF under night break treatment (LIGHT) and dark night control (No Light) in six clones. B-Mean number of DTF under PGR and pruning treatment (Hormone) and no hormone and pruning control (Control) in six four clones.

fruits, and seeds in four CBSD-resistant or immune clones in Kenya. These results are consistent with experiments conducted in Nigeria (Oluwasanya et al., 2021) and Colombia (Hyde et al., 2020). This breakthrough will dramatically increase the number of crosses that can be made in the cassava breeding program, thereby increasing the efficiency of breeding, particularly for CBSD resistance.

Ethylene, a plant hormone, affects various developmental processes, including seed germination, fruit ripening, senescence, abscission, and stress responses (Abeles, 1992). Silver ions, such as those supplied by STS, bind to ethylene receptors, reducing ethylene binding and effectiveness (Dar and Tahir, 2018). STS provides silver as a mobile and persistent ionic complex in plant tissue, binding to ethylene receptors and preventing responses to ethylene, thereby increasing flower longevity (Dar and Tahir, 2018). This mechanism likely explains the reduced flower abortion observed in treated clones DSC 248 and DSC 257 compared to the untreated controls.

Hyde et al. (2020) also observed that STS increased flower longevity in cassava and promoted flower production by prolonging flower bud formation, ultimately increasing the number of flowers formed. This finding explains the increased number of female flowers observed in the PGR treatment described here relative to the non-treated controls. Notably, despite STS application, DSC 120 aborted the inflorescence at the second tier, indicating that responses to PGRs may be dose- and genotype-dependent. Consequently, experiments need to be performed on each genotype to obtain the desired response for breeding. Similar observations were made by Kupke et al. (2022) while manipulating barley for flowering enhancement using PGRs.

BA, a first-generation synthetic cytokinin influences cell division which affects plant growth and development, particularly physiological processes such as germination, flowering, seed development, and leaf senescence (Hönig et al., 2018). It has been shown to increase the proportion of female flowers in cassava (Oluwasanya et al., 2021, Baguma et al., 2023); (Hyde et al., 2020). BA treatment has also been utilized in promoting floral feminization and fruiting in the oil seed crop *Plukenetia volubilis* (Fu et al., 2014), biofuel shrub (*Jatropha curcas)* (Liu et al., 2019, Pan and Xu, 2011) and date palm (*Phoenix dactylifera*) (Ashour et al., 2018). The larger proportion of female flowers observed here (Figure 2) can be partly attributed to the effects of BA. Pruning of the young branches has been shown to strengthen the apical dominance of the terminal inflorescence and in this way reduces abortion (Pineda et al., 2021). In the current study pruning was used together with PGR to both

Table 4. Variance analysis for effects of night breaks on flowering parameters in six clones.

Source of variation	df	Number of female flowers	Number of fruits	Number of nodes to 1st branching	Shoot weight	Root weight	Plant height to 1st branching	Days to 1st flowering
Replicates	11	0.21	0.10	0.12	0.01	0.02	3.34	9.78
Condition	1	6.37***	1.01***	0.37***	0.33***	0.42***	2360.18***	38841.84***
Clones	5	6.15***	0.80***	0.75***	0.23***	0.12***	7986.12***	66081.09***
Condition × Clones	5	3.79***	0.59***	2.43***	0.01***	0.04**	7479.16***	30747.26***
Error	121	0.24	0.06	0.12	0.02	0.01	4.00	6.61
R^2		0.67	0.56	0.56	0.54	0.54	0.99	0.99
CV (%)		17.68	9.78	10.33	4.94	3.93	2.96	2.10

*,**,*** =significant at p<0.05, 0.01 and 0.001, df =degree of freedom, CV (%) =coefficient of variation, R^2=coefficient of determination

Table 5. Pearson correlation coefficient of agronomic traits of 6 cassava clones under night break extended photoperiod.

Traits	Number of flowers	Number of fruits	Number of nodes	Shoot weight	Root weight	Plant height to first branching
Number of fruits	0.94***					
Number of nodes	0.54	0.56				
Shoot weight	0.13	0.33	0.70			
Root weight	0.26	0.31	0.58	0.47		
Plant height to first branching	0.33	0.34	0.95***	0.69	0.37	
Days to flowering	-0.10	-0.30	0.44	-0.05	0.05	0.61

, * =significant at p<0.01 and p<0.001.

increase the number and proportion of female flowers. The observations made in this study are consistent with Oluwasanya et al. (2021), where pruning was applied with or without BA and STS and increased the number of female flowers and the number of flowers and fruits. A strong and positive correlation was found here between flowering, number of fruits and seeds (Table 3), which is consistent with other studies on cassava (Baguma et al., 2023). Studies of Euphorbiaceae-family have shown that when applied separately, pruning (Pineda et al., 2021), STS (Hyde et al., 2020) and BA (Fu et al., 2014) affect flower development by predominantly but not exclusively increasing flower longevity, reducing abortion and feminizing flowers respectively. Studies by Oluwasanya et al. (2021) demonstrated that a combination of plant growth regulators, pruning, and extended photoperiod substantially improved reproductive development in cassava. This treatment combination increased the number of female flowers and fruits, corroborating the findings of this current study. Collectively, these treatments can be used to increase the number of female flowers, prolong flowering longevity, and reduce abortion rates in Kenya, depending on the clone. This, in turn, enhances the efficiency of cassava breeding programs.

Night break in induction of flowering

Asynchronous flowering poses significant challenges to breeders aiming to cross specific genotypes, including those with CBSD resistance. Previous studies have reported that extended photoperiods using red light and night breaks effectively induce early flowering in cassava (Pineda et al., 2021; Santos et al., 2023; Baguma et al., 2023). This study investigated whether a cost-effective method of interrupting the length of darkness at night through night breaks, using readily available white light in Kenya, could help synchronize flowering.

Induction of flowering in six cassava genotypes was undertaken by night break (NB) using 100W LED white light (WL). Night break, or night interruption, involves applying a short period of light during the dark phase of the night. This technique has been utilized to reduce the number of consecutive hours of darkness, as cassava is a long-day plant that flowers in response to long days and short nights (Pineda et al., 2021; Adeyemo et al., 2019).

Temperature has been shown to interact with photoperiodism, where high temperatures inhibit flowering and cool temperatures promote flowering under extended photoperiodism (Hyde and Setter, 2022; Adeyemo et al., 2019). The experiment was conducted at an altitude of 1548 m above sea level (masl), providing ambient temperatures ranging from 21 to 28°C. This temperature range is within the suggested optimal range for breeding nurseries to be supplemented with extended photoperiod, between a maximum of ≤30°C and a minimum of >15°C, to induce and enhance flowering (Rodrmguez et al., 2023).

The results showed that night breaks using white light reduced the number of days to flowering in all clones. This technique can thus be used to synchronize flowering in different cassava genotypes in Kenya, extending the range of genotypes that can be crossed by breeders. This will be extremely useful in breeding for CBSD tolerance, enabling the crossing of genotypes from South America and Africa. Furthermore, this study demonstrates that white light can be effectively used in place of red light, which is conventionally used. This opens up the application of this technology to areas where red lights are not easily available.

Extended photoperiod can also be utilized as a speed breeding technique (Watson et al., 2018), aiming to reduce and shorten breeding cycles by increasing generation cycles, thereby accelerating crop improvement. In cassava, similar results have been reported by Baguma et al. (2023), Pineda et al. (2021), Adeyemo et al. (2019), Santos et al. (2023), and Rodrmguez et al. (2023), who observed a reduced number of days to flowering in some genotypes from 6-7 months to 3-4 months. Similar effects have been reported in other crops, such as chickpea (*Cicer arietinum L*.) (Samineni et al., 2020) and *Arabidopsis thaliana* (Goto et al., 1991).

The results of this study showed that night breaks enhanced flowering by increasing the number of female flowers and fruits, compared to the control, in all genotypes except DSC272. In DSC272, the abortion rate was higher under the night break experiment, resulting in fewer flowers compared to the control. This study corroborates the findings of Baguma et al. (2023), who reported that night breaks enhance the number of female flowers and fruits.

Plant architecture was also affected by night breaks, as flowering was accompanied by branching or forking in all clones under the night break experiment. In contrast, DSC 196 did not branch or flower in the dark night experiment. The height to first branching and the number of nodes to first branching were reduced in the night-break experiment compared to the control.

Experiments conducted in this study demonstrate that PGRs and pruning can be used to increase flowering and the proportion of female flowers in Kenya. This can be used to increase the number of crosses that can be performed, thereby increasing the efficiency of breeding. Extended photoperiod, applied through white light and night breaks, can both induce earlier and enhance flowering. This can be used to synchronize flowering, extending the range of genotypes that a breeder can cross.

The technology is effective at locations close to the equator with minimal natural shift in photoperiod. The use of white lights provides opportunities for the application of these technologies in places where red lights are not easily available. It is recommended that these methods be used together in a single treatment in breeding nurseries. However, response to PGRs and extension of photoperiodism is genotype-dependent; therefore, it is recommended to screen genotypes for response before planning crosses.

Conclusions

The study indicates that PGRs, pruning and extended photoperiodism can increase the number of female flowers of cassava in Kenya and induce earlier flowering. The combination of PGR, pruning and extension of photoperiod is applicable in breeding fields and is amenable to use in off-grid conditions.

CONFLICT OF INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. Means of flowering parameters for four clones under treatment (PGR and pruning) and no-treatment control

Clone	TRT	No_of_female_flowers_mean	No_of_fruits_mean	Height to first branching_mean	DTF_mean	No_of_Seeds_mean
DSC 248	Ctrl	8.75	1.4	92.25	187.35	2.8
DSC 248	Treatment	21.9	10.05	92.55	163.55	16.3
DSC 251	Ctrl	7.9	3.65	86.45	61.1	8.2
DSC 251	Treatment	22.9	10.35	86.95	57.55	20.8
DSC 257	Ctrl	8.95	1.75	72.4	119.55	3.6
DSC 257	Treatment	18.45	8.3	72.05	106.95	17.6
DSC 258	Ctrl	4.55	1.4	103.85	216.65	3.25
DSC 258	Treatment	11.9	5.3	102	207.5	11.95

Supplementary Table 2. Means of flowering traits for six clones under night break extended photoperiod and dark-night control (No Light)

Condition	Clone	Height to first branching	Root weight mean	Shoot weight mean	Nodes to first br mean	No of fruits mean	No_of_flowers_mean
LIGHT	DSC 120	63.19444444	4.420417	1.809722	15.22222	16.22222	155.0833333
LIGHT	DSC 196	62.30555556	4.570278	0.409722	26.27778	3.444444	6.388888889
LIGHT	DSC 257	59.83333333	2.959028	2.961806	15.66667	5.472222	28.08333333
LIGHT	DSC 258	57.61111111	4.985333	2.11125	15.97222	0.361111	1.5
LIGHT	DSC 269	68.38888889	4.325972	1.262056	16.44444	0.222222	0.972222222
LIGHT	DSC 272	68.91666667	5.265889	1.936806	18.13889	2.472222	5.611111111
NO LIGHT	DSC 120	92.22222222	4.294306	2.790694	30.5	2.111111	5.972222222
NO LIGHT	DSC 196	0	1.901111	1.416944	0	0	0
NO LIGHT	DSC 257	86.80555556	1.932083	3.284444	23.30556	2.555556	7.055555556
NO LIGHT	DSC 258	80.88888889	3.170972	2.96625	23.16667	0	0
NO LIGHT	DSC 269	76.05555556	1.765556	0.999028	21.72222	0	0
NO LIGHT	DSC 272	92.86111111	5.036111	8.073972	26.58333	4.833333	10.91666667