http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.369.379

CrossMark

# Research Article
# Correlation Between Relative Gene Expression Patterns of Two *Flowering locus T* (*MeFT1* and *MeFT2*) in Cassava Leaf and Flowering Traits Under Different Flowering Induction Conditions

[1]Salilthip Leelawijitkul, [1]Pasajee Kongsil, [1]Piya Kittipadakul and [2]Piyada Juntawong

[1]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

## Abstract
**Background and Objective:** *Flowering locus T* (*FT*) genes are involved in the flower induction mechanism in plants as florigen signals. The objective of this study was to study the relationship between the expression of *Flowering locus T* genes (*MeFTs*) in cassava and flowering traits under the different flowering induction conditions. **Materials and Methods:** The experimental design for flowering induction was RCBD for 4 replications. There were 5 treatment factors which were control, red light set from 5 pm to 7 am, 0.5 mM 6-benzyladenine (BA) with 2 mM silver thio-sulfate (STS), paclobutrazol for 6 g/plant and potassium chlorate ($KClO_3$) for 250 g/plant. The number of plants with flower bunches and the average number of bunches per plant in two cassava varieties were collected each month from 5-9 months after planting (MAP). The leaf samples were collected from HB80 and R9 varieties at 5-7 MAP for RNA extraction to study *MeFT1* and *MeFT2* expression. **Results:** The results show that *MeFT1* expression level positively correlated with flowering traits in the same month, while *MeFT2* expression level positively correlated with flowering traits in the following months. **Conclusion:** Therefore, expression of *MeFT2* can be used for the prediction of cassava flowering in the following month which will assist the breeder for the crossing management.

Key words: Cytokinin, flowering bunches, paclobutrazol, photoperiod extension, potassium chlorate

Citation: Leelawijitkul, S., P. Kongsil, P. Kittipadakul and P. Juntawong, 2022. Correlation between relative gene expression patterns of two *Flowering locus T* (*MeFT1* and *MeFT2*) in cassava leaf and flowering traits under different flowering induction conditions. Pak. J. Biol. Sci., 25: 369-379.

Corresponding Author: Pasajee Kongsil, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Cassava is one of the tropic crops which has increasing importance in industries and food security[1]. In terms of nutrition, cassava is an important source of carbohydrates. About 18-33% of starch by fresh weight was reported in the root of Thai cassava varieties[2]. The main problems of cassava are biotic stresses and drought. So, the cassava breeding programs focus on high yield and starch contents along with resistance to diseases, insects and various environmental problems, particularly drought. Specific crosses among outstanding cassava varieties used as progenitors are the critical first step in the breeding process. However, the chosen cassava varieties used as progenitors have flowering problems due to their erect plant architecture since cassava flowering bunches occurred at the forking point of each branch level. Farmer prefers unbranching types which are, by default, late-flowering varieties (often these varieties fail to produce a flower or they require more than 12 months after planting to flower in crossing nurseries). The scarcity of flowering in erect phenotypes is further complicated by difficulties in the synchronization of flowering from different genotypes.

Cassava is diploid (2n = 36)[3], highly heterozygous and can be vegetatively propagated through stakes in agriculture[4]. Typical of outcrossing species cassava show inbreeding depression for several trait[5]. Cassava is monoecious plant. It flowers earlier in long-day photoperiod than in short-day photoperiod[6]. Inflorescences have pistillate (female) flowers at the base and staminate (male) flowers at the top. Synchronization of female and male flowering can be achieved from inflorescences in different branches[7].

To induce flowering, illumination with red light during the night[8] and 6-benzyladenine (BA) with silver thio-sulfate (STS)[9,10] have been successfully used to induce the flowering of cassava. Cytokinin increases in the process of dividing cells of various organs and be used to speed up flowering time and prevents the reduction of chlorophyll. 6-Benzyl aminopurine (BA) is a synthetic cytokinin and STS is commonly used to inhibit the production of ethylene used in flowering induction in cassava[10]. Paclobutrazol (PBZ) is often used to regulate the growth of plants, resulting in reduced plant cell size[11]. In general, PBZ can restrain Gibberellin synthesis[12]. The PBZ inhibits cell elongation in the subapical meristem area and did not affect the leaves which were at the apex[13]. As a result, the length of the branch length of the plant was reduced and it stimulated the flower production (earlier and/or profuse flowering) of many plant species[14]. The PBZ was applied to induce or enhance flowering in cassava, but there was no interaction effect between PBZ and $KNO_3$ on the vegetative growth and induction of flowering[15]. Another plant growth regulator is potassium chlorate ($KClO_3$) which can be used to stimulate off-season longan flowers. The $KClO_3$ is a strong oxidizer or oxygen filler[16].

During the process of flowering in Arabidopsis, the *Flowering locus T* (*FT*) gene expression was found in the leaf. When *FT* mRNA was transferred to the apical meristem, it induced other gene expressions and then leads to a flowering. For this reason, *FT* mRNA level is a key component in the florigen signaling[17]. The *FT* genes are also involved in cassava flowering[6]. Overexpression of *FT* gene from Arabidopsis in cassava hastened flower initiation in cassava[18,19]. *Flowering Locus T* genes in cassava were named *MeFT1* and *MeFT2* and were located at chromosomes 12 and 13 of cassava, respectively[6]. It has been reported that early-flowering cassava varieties have a higher expression level of *MeFT1* than those of late-flowering ones[9].

The objectives of this study were to analyze the relationship between the expression of *Flowering locus T* (*FT*) genes and cassava flowering traits of Huay Bong 80 (HB80) and Rayong (R9) varieties, which were representative of early and late flowering variety respectively, under different induction conditions and to assess the potential of evaluating *MeFT* gene expression level as a precise and early physiological indicator of plant response to flowering induction treatments.

## MATERIALS AND METHODS

**Plant materials and flowering induction treatment:** The experiment was conducted at the Tapioca Development Institute (TDI), Huay Bong district, Nakhon Ratchasima province, Thailand at 325 m above sea level. Stem cuttings were planted in June, 2019. The experimental design was in RCBD with four replications. There were four flowering induction treatments which were extended photoperiod exposure with 50 Watt red light-emitting diode (LED) hanged 3 m above ground covering light for 9 plants in diameter of each spotlight from 5 pm to 7 am starting 45 days after planting until the end of the flowering period, 0.5 mM 6-benzyladenine (BA) and 2 mM silver thio-sulfate (STS) applied twice a month via foliar spraying, PBZ at the rate of 6 g/plant and $KClO_3$ at the rate of 250 g/plant. The last three treatments were taken place at 4 months after planting (MAP). The data were analyzed a per set of cassava varieties: Huay Bong 80 (HB80) which was early flowering and Rayong9 (R9) which was late flowering. Chemical fertilizer 15-15-15 at the rate of 312.5 kg $ha^{-1}$ was applied at 1 MAP. Hand weeding was made three times throughout the rainy season. The flowering traits monthly collected during the flowering period from 5-9 MAP were the number of plants with flower bunches (Pl-B) and the average number of bunches per plant (B). The number of male (M) and female (F) flowers per bunch were also counted to estimate the quality of flowering bunches. Branching levels were evaluated at 4, 5 and 9 MAP.

The weather station collected rainfall and air temperature data as follows. The rainfall occurred between May (the beginning of the planting season) and October, 2019 which had a monthly average rainfall between 38.90-188.40 mm. From December, 2019 to February, 2020, there was no rainfall. The rain started again in March and April, 2020 with a monthly average of 25.30 to 80.40 mm. The average monthly temperature throughout the experiment reached the highest in March, 2020 at 37.01 °C and the lowest in December, 2019 at 19.03 °C.

***FT* gene expression study:** Cassava mature leaves were monthly collected only from HB80 and R9 (representative variety of early and late flowering, respectively) from 5-7 MAP in the collection period around 2-3 pm. mRNA was isolated from leaf samples to study *MeFT1* and *MeFT2* gene expression levels. Total RNA isolation was performed by a standard protocol[20]. Total RNA was treated with RNase-free DNase I (Thermo Scientific TM) for 5 mg/sample. Total DNA-free mRNA in the amount of 1 mg was used for cDNA synthesis using the Oligo dT primer method of Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific TM). Quantitative PCR (qPCR) was performed using a Helixis 5421 (Avenida Encinas, California, USA) with SYBRTM Green Master Mix (Thermo Scientific TM). The qPCR analysis was performed using the standard curve method of five-fold dilution series of pooled cDNA of all samples as standard. The ubiquitin 10 gene ($UBQ_{10}$) was used as a reference gene. Gene-specific primer sequences of *Flowering locus T* genes (*MeFT1* and *MeFT2*) and $UBQ_{10}$ gene as house-keeping genes were obtained from[6]. The relative expression was measured using the reference gene-normalized expression data of the cDNA sample of R9 leaf under control condition in the first replication to divide another normalized expression of the other samples. The expression level data were transformed into $Log_2$ for statistical analysis.

**Statistical analysis:** Mean and standard deviation of the number of plants with flowering bunches (Pl-B), the number of bunches per plant (B), the $log_2$ transformed values of *MeFT1* and *MeFT2* gene expression were calculated from data of four replications. To reveal the qualitative data of Pl-B and B, the total sum of values and the range of values in all replications were shown together with the range of the number of male and female flowers per bunches. Correlation analysis was performed using Pearson's correlation analysis by STAR program (IRRI).

## RESULTS AND DISCUSSION

For indicating the response of plants to flowering induction treatments, the number of plants with flower bunches (Pl-B) and the average number of bunches per plant (B) were cumulatively collected and summed into monthly data for analysis. There were five plants per experimental unit. Therefore, the maximum number of plants with flower bunches should be five. Most cassava in this experiment had a flowering period from 5-9 months after planting (MAP). Table 1 and 2 explain the trend of flowering of HB80 and R9, respectively. The branching levels (Br) were counted at 4 MAP which was the time before flowering, at 5 MAP which was the first month of flowering of some varieties under some conditions and then at 9 MAP which was the last month of cassava flowering in this location. The number of male (M) and female (F) flowers were counted per bunch as shown in the range of the number per bunch for indicating the quality of flowering bunches in this study.

Huay Bong 80 (HB80) is the variety which has moderately branches[1]. Therefore, the increasing number of bunches of flowers was expected as the age of plant increase as reported in other branched-type cassava, Kasetsart50, reported in Tokunaga *et al.*[21]. In Table 1, HB80 under red light tended to accelerate branching faster than other conditions at the 4-5 MAP period. All plants under red light treatment showed at least 2 branching level at 5 MAP (Br = 2-3 at 5 MAP), while those under other treatments showed the least branching level from 0-1 at 5 MAP (Br = 1-3 under control, Br = 1-3 under BA+STS, Br = 0-2 under PBZ, Br = 1-2 under $KClO_3$). However, at 9 MAP, the level of branching in plants under all conditions reached the level of 2-3 branching. The numbers of plants with flowering bunches of HB80 in response to flowering induction treatments were around 0-1 plants of the total of five plants. Under control conditions and BA+STS, HB80 had a flowering period from 5-9 MAP. The numbers of flowering plants under control were reduced from 1-0.25 plants within the period from 5-9 MAP. The average number of flowering plants under BA+STS reached the peak of 1 plant at 7 MAP. Under red light, the flowering period was from 5-7 MAP (Pl-B = 0.75 each month) and then again at 9 MAP (Pl-B = 0.50 at 9 MAP). Under PBZ and $KClO_3$, HB80 had a flowering period from 8-9 MAP (Pl-B = 0.5 each month for PBZ and Pl-B

Table 1: Number of plants with flower bunches (Pl-B), the average number of bunches per plants (B), the range of level of branching (Br) and the range of the number of male (M) and female (F) flower per bunch in HB80 responded to induction treatments from 5-9 months after planting

| | 5 MAP | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range |
| Control | 1.00±0.82 | 4 (0-2) | 1.00±0.82 | 8 (0-3) | 0.75±1.50 | 3 (0-3) | 0.25±0.50 | 1 (0-1) | 0.25±0.50 | 0 (0-1) |
| BA+STS | 0.75±0.96 | 3 (0-2) | 0.75±0.96 | 5 (0-4) | 1.00±0.82 | 4 (0-2) | 0.25±0.50 | 1 (0-1) | 0.75±0.96 | 3 (0-2) |
| Red light | 0.75±0.50 | 3 (0-1) | 0.75±0.50 | 11 (0-5) | 0.75±0.96 | 3 (0-2) | 0.00±0.00 | 0 | 0.50±0.58 | 2 (0-1) |
| PBZ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.50±0.58 | 2 (0-1) | 0.50±0.58 | 2 (0-1) |
| $KClO_3$ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.25±0.50 | 1 (0-1) | 1.00±1.41 | 4 (0-3) |

| | 5 MAP | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | B | Total: Range | B | Total: Range | B | Total: Range | B | Total: Range | B | Total: Range |
| Control | 1.00±0.82 | 5 (1-2) | 5.75±5.09 | 66 (1-20) | 1.50±3.00 | 18 (5-8) | 1.5±3.00 | 6 (6) | 3.00±6.00 | 12 (12) |
| BA+STS | 1.00±1.15 | 6 (1-3) | 3.44±4.33 | 28 (1-9) | 7.75±7.72 | 42 (3-19) | 4.25±8.50 | 17 (17) | 7.00±9.45 | 36 (7-20) |
| Red light | 1.00±0.82 | 4 (1-2) | 3.88±2.69 | 55 (1-13) | 3.88±7.10 | 30 (1-19) | 0.00±0.00 | 0 | 4.00±4.69 | 16 (7-9) |
| PBZ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 5.25±6.08 | 21 (10-11) | 5.00±8.12 | 20 (3-17) |
| $KClO_3$ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 2.25±4.50 | 9 (9) | 8.17±11.17 | 80 (9-33) |

| | 4 MAP | 5 MAP | | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Br | M | F | Br | M | F | M | F | M | F | M | F | Br |
| Control | 1 | 3 | 2-3 | 1-3 | 5-89 | 1-6 | 12-94 | 1-4 | 2-20 | 5 | 8-24 | 1-2 | 2-3 |
| BA+STS | 1 | 18-83 | 2-3 | 1-3 | 24-105 | 1-15 | 8-55 | 1-4 | 10-35 | 1-6 | 7-32 | 1-12 | 2-3 |
| Red light | 2 | 57 | 2-3 | 2-3 | 10-80 | 1-12 | 5-38 | 1-3 | 0 | 0 | 6-35 | 1-4 | 2-3 |
| PBZ | 0-1 | 0 | 0 | 0-2 | 0 | 0 | 0 | 0 | 32-52 | 1-4 | 5-18 | 1-4 | 2-3 |
| $KClO_3$ | 0-2 | 0 | 0 | 1-2 | 0 | 0 | 0 | 0 | 25-35 | 2-4 | 3-74 | 1-3 | 2-3 |

The total number of flowering plants were shown followed by the range of the number of flowering plants in all replications (Total: Range). The total number of bunches were shown followed by the range of the number of flower bunches per plant in all replications (Total: Range). The range of level of branching in all replications was shown in the column named 'Br'. The range of the number of male and female flowers per bunch in all replications was shown in the column named 'M' and 'F', respectively

Table 2: Number of plants with flower bunches (Pl-B), the average number of bunches per plants (B), the range of level of branching (Br) and the range of the number of male (M) and female (F) flower per bunch in R9 responded to induction treatments from 5-9 months after planting

| | 5 MAP | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range | Pl-B | Total: Range |
| Control | 1.00±1.41 | 4 (0-3) | 0.50±0.58 | 2 (0-1) | 0.50±1.00 | 2 (0-2) | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| BA+STS | 0.25±0.50 | 1 (0-1) | 1.00±1.41 | 4 (0-3) | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| Red light | 0.00±0.00 | 0 | 0.25±0.50 | 1 (0-1) | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| PBZ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| $KClO_3$ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.50±0.58 | 2 (0-1) |

| | 5 MAP | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | B | Total: Range | B | Total: Range | B | Total: Range | B | Total: Range | B | Total: Range |
| Control | 0.50±0.58 | 4 (1) | 0.75±0.96 | 3 (1-2) | 0.75±1.50 | 6 (2-4) | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| BA+STS | 0.25±0.50 | 1 (1) | 1.75±2.06 | 13 (1-7) | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| Red light | 0.00±0.00 | 0 | 1.75±3.50 | 7 (7) | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| PBZ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 |
| $KClO_3$ | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 0.00±0.00 | 0 | 6.75±8.06 | 27 (11-16) |

| | 4 MAP | 5 MAP | | | 6 MAP | | 7 MAP | | 8 MAP | | 9 MAP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Br | M | F | Br | M | F | M | F | M | F | M | F | Br |
| Control | 0 | 0 | 1-3 | 1-3 | 10-15 | 1-2 | 10-40 | 3-4 | 0 | 0 | 0 | 0 | 1-3 |
| BA+STS | 0 | 0 | 9 | 1-3 | 15-70 | 1-8 | 0 | 0 | 0 | 0 | 0 | 0 | 1-3 |
| Red light | 0 | 0 | 0 | 0-1 | 30-40 | 1-6 | 0 | 0 | 0 | 0 | 0 | 0 | 1-2 |
| PBZ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0-1 |
| $KClO_3$ | 0-1 | 0 | 0 | 0-1 | 0 | 0 | 0 | 0 | 0 | 0 | 6-30 | 1-5 | 0-3 |

The total number of flowering plants were shown followed by the range of the number of flowering plants in all replications (Total: Range). The total number of bunches were shown followed by the range of the number of flower bunches per plant in all replications (Total: Range). The range of level of branching in all replications was shown in the column named 'Br'. The range of the number of male and female flowers per bunch in all replications was shown in the column named 'M' and 'F', respectively increased from 0.25-1.00 from 8-9 MAP for $KClO_3$). Under control conditions, HB80 had the peak of flowering bunches per plant at a 6 MAP averagely of around 5.75 bunches (the total bunches summed from five plants were 66 bunches ranging from 1-20 bunches per plant). Under BA+STS, HB80 had the peak of flowering bunches per plant at 7 and 9 MAP averagely 7 and 7.75 bunches, respectively. At 7 MAP, the total bunches summed from five plants were 42 bunches ranging from 3-19 bunches per plant, while, the total bunches summed from five plants were 36 bunches ranging from 7-20 bunches per plant at 9 MAP. Under red light treatment, HB80 had the peak of flowering bunches per plant at 6-7 MAP and 9 MAP around 3.88 to 4 bunches. Under PBZ, HB80 had bunches around 5-5.25 bunches during the last two-month flowering period, while, under $KClO_3$, the peak of flowering bunches occurred at 9 MAP around 8.17 bunches. The number of male and female flowers per bunches was the highest at 6 MAP under control, BA+STS and red light. The number of male flowers ranged from 24-105 flowers per bunch and the number of female flowers ranged from 1-15 flowers per bunch under BA+STS which were higher than those under control (M = 5-89, F = 1-6) and red light (M = 10-80, F = 1-12) conditions. Considering the total number of bunches and flowering plants which accumulated from all months of the flowering period, HB80 under BA+STS condition had the better potential of flowering than those under induction conditions even though red light tended to induce more branching level at the early stage around 4-5 MAP. The summed number of flowering bunches within the five months was the highest in BA+STS which were 129 bunches from all plants under treatment as calculated from 6, 28, 42, 17 and 36 total bunches from 5-9 MAP, respectively, while those under the red light were 105 bunches from all plants under a red light as calculated from 4, 55, 30, 0 and 16 total bunches from 5-9 MAP, respectively. Therefore, the branching induction might not correlate with the potential of flowering in HB80.

Rayong 9 (R9) is a straight-plant type cassava[1]. In Table 2, plants under control and BA+STS tended to develop branching levels (ranging from 1-3 level at 5 MAP) faster than other conditions (ranging from 0-1 level at 5 MAP). In contrast with the result in HB80, the red light tended to reduce the branching potential of R9 (ranging from 0-1 level at 5 MAP) compared with the control group. The numbers of plants with flowering bunches of R9 in response to flowering induction treatments were averagely 0-1 plants of the total of five plants. Under control conditions, R9 had a flowering period from 5-7 MAP which was longer than under other conditions. The number of flowering plants under control decreased from 1.0-0.5 plants during the 5-7 MAP period. Under BA+STS treatment, the flowering period was from 5-6 MAP in which there were 0.25 flowering plants at 5 MAP and 1.0 flowering plants at 6 MAP; while, under red light treatment, the flowering period was only at 6 MAP in only average of 0.25 flowering plants. There was no plant with flowers under Paclobutrazol (PBZ) treatment and there were only 0.5 flowering plants at 9 MAP under $KClO_3$ treatment. The average number of bunches per plant increased from 0.5-0.75 bunches per plant from 5 MAP to 6 and 7 MAP under control (the total bunches summed from five plants was 13 bunches as calculated from 4, 3 and 6 bunches from 5-7 MAP, respectively). Under BA+STS, the average number of flowering bunches per plant increased rapidly from 0.25-1.75 from 5-6 MAP (the total bunches summed from five plants was 14 bunches as calculated from 1 and 13 bunches from 5-6 MAP, respectively). There was only a one-month period of flowering at 6 MAP under the red light. The average number of flowering bunches per plant was 1.75 (the total bunches summed from five plants were 7 bunches). Interestingly, even though $KClO_3$ caused a late flowering period in R9, the number of flowering bunches was the highest among all treatments throughout a whole flowering period. The average number of flowering bunches per plant under was around 6.75 under $KClO_3$ treatment (the total bunches summed from five plants were 27 bunches) at 9 MAP. Thus, considering the total number of bunches which accumulated from all months of the flowering period, R9 under control condition (total 13 bunches) and BA+STS (total 14 bunches) had the better potential of flowering than those under other induction conditions during the early flowering period even though there were more flowering bunches for plants under BA+STS than under control at 6 MAP. However, if the flowering crossing was planned at the late flowering period, the induction with $KClO_3$ is effective for flowering induction of R9 with the highest flowering potential. There was no flower in R9 under any treatment conditions in 8 MAP. The number of male and female flowers per bunches was the highest at 6 MAP for R9 under BA+STS (ranging from 15-70 flowers per bunch for male flowers and 1-8 flowers per bunch for female flowers) and red light (ranging from 30-40 flowers per bunch for male flowers and 1-6 flowers per bunch for female flowers) conditions, while, those under control condition reached the peak at 7 MAP (ranging from 10-40 flowers per bunch for male flowers and 3-4 flowers per bunch for female flowers).

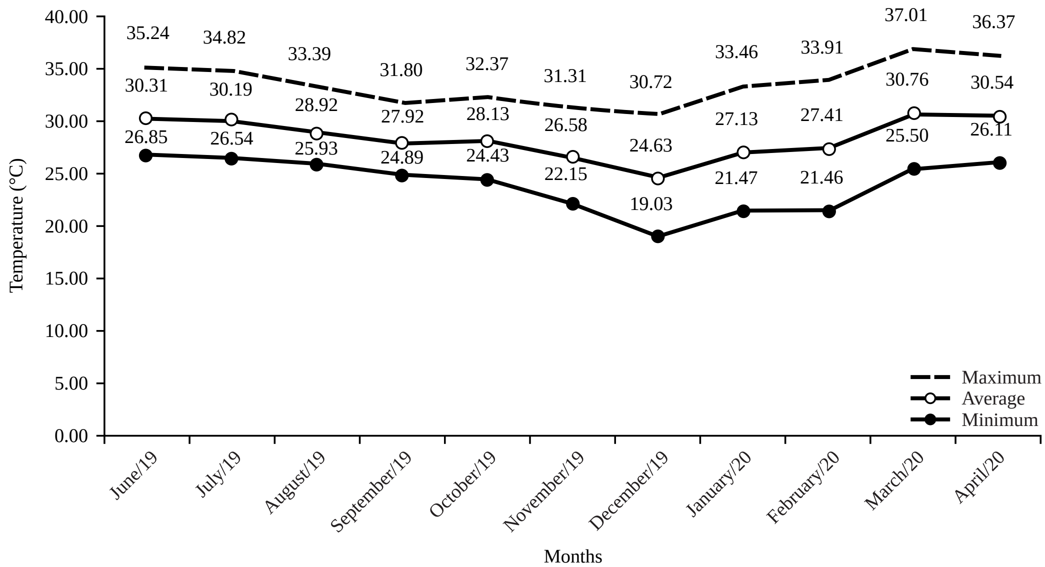


Fig. 1: Air temperature throughout the growing season at the experimental site, TDI, Huay Bong

In this experiment, the flowering period had a range from 5-9 MAP and occurred abundantly at 6 MAP when the temperature was the lowest and averagely at 19.03°C at night and averagely at 24.63°C in December, 2019 in this experimental location. The temperature decreased gradually from the planting date in June, which had the maximum and minimum temperature at 35.24 and 26.85°C, respectively. Until the first month of the flowering period in November, the maximum and minimum temperatures were at 31.31 and 22.15°C, respectively. Then, the temperature increased again in March when the maximum and minimum temperatures at 37.01 and 25.50°C, respectively in Fig. 1 in the similar quantity of flower bunches reported in Perera *et al.*[7] around 3.9-4.9 bunches. Souza *et al.*[22] reported that most cassava flowering occurs during the longest photoperiod around 5-6 MAP in EMBRAPA experimental site where the average temperature was around 22-25°C. Ibrahim *et al.*[23] also reported the first flowering period around 4-7 MAP. Compared to the cassava flowering period in our studies with others' studies, the flowering period in this study was a little bit delayed from others' studies. Adeyemo *et al.*[6] has suggested the optimum range of temperature for cassava flowering initiation from 22-34°C. Therefore, a bit warmer temperature in this study could delay the flowering initiation period. Oluwasanya *et al.*[24] also suggested that the late-flowering genotypes tend to be sensitive to an unfavourable condition which had resulted in delaying of flowering time. For this reason, the red-light extension and BA+STS treatments in this study did not significantly affect on fasten flowering time in both early and late flowering genotypes which was in contrast to the result of Pineda *et al.*[8] that extend photoperiod by red light could fasten flowering time of erect genotypes which were late flowering types in a location having temperature ranged from 19.2-30.1°C. In addition, anti-ethylene silver thiosulfate (STS) was reported to increase flower abundance in cassava[10], but BA+STS treatment in this experiment did not significantly induce more flower bunches even though it tended to increase the amount of flower bunch in HB80 variety compared to the control condition. However, the treatment method of STS in this study was different from Oluwasanya *et al.*[10] since the strongly windy condition in the experimental field caused unsuccessful in the installing of petiole feeding of STS solution. Therefore, spraying with a higher concentration of STS was our solution of STS application which might cause the difference in the result. Moreover, the growth retardant as Paclobutrazol and $KClO_3$ in this experiment seemed to delay the flowering period for cassava. This result was against the hypothesis that growth retardants such as PBZ and $KClO_3$ would reduce vegetative growth and switch to flower initiation. It might be due to the negative effect of these growth retardants to plant growth not only in the vegetative part which also had an indirect effect on plant development into other stages as well. However, the number of flowering bunches undergrowth retardant induction were considerable profound for crossing plan in the late breeding season (from 8-9 MAP). The total number of flower bunches in HB80 under PBZ was 41 bunches (21 bunches at 8 MAP and 20 bunches at 9 MAP) and under $KClO_3$ was 89 bunches (9 bunches at 8 MAP and 80 bunches at 9 MAP), while, those of R9 under $KClO_3$ was 27 bunches.

Table 3: Relative gene expression level in $log_2$ transformed values of *MeFT1* and *MeFT2* in leaf samples of HB80 and R9 responded to induction treatments from 5-7 MAP

| Gene expression ($Log_2$ transformed) | *MeFT1* | | | | *MeFT2* | | | |
|---|---|---|---|---|---|---|---|---|
| | HB80 | Range | R9 | Range | HB80 | Range | R9 | Range |
| **5 MAP** | | | | | | | | |
| Control | 0.89±1.68 | 2 (-0.48, 3.26) | 0.65±1.23 | 2 (-0.52, 2.28) | 2.81±2.11 | 4 (0.30, 5.47) | 0.47±0.38 | 3 (0, 0.94) |
| BA+STS | 1.01±2.53 | 3 (-1.95, 4.21) | 2.89±1.20 | 4 (1.52, 4.09) | 2.08±1.22 | 4 (1.09, 3.75) | 0.81±2.10 | 3 (-1.76, 3.38) |
| Red light | 1.15±1.67 | 3 (-0.37, 3.52) | 2.44±2.70 | 3 (-0.44, 5.21) | 1.71±1.54 | 4 (0.50, 3.73) | 3.79±0.48 | 3 (3.20, 4.38) |
| PBZ | 1.70±2.00 | 3 (-1.15, 3.51) | 2.11±3.45 | 3 (-2.64, 4.86) | 2.36±0.00 | 1 (2.35) | 2.01±2.32 | 3 (-0.77, 4.91) |
| $KClO_3$ | -0.50±3.12 | 2 (-4.29, 3.22) | 1.82±2.55 | 3 (-1.59, 4.49) | 1.60±2.32 | 3 (-1.27, 4.40) | 5.49±0.14 | 3 (5.32, 5.66) |
| **6 MAP** | | | | | | | | |
| Control | -0.09±0.90 | 1 (-1.03, 1.13) | -0.01±0.97 | 1 (-0.74, 1.36) | -0.79±1.26 | 1 (-2.03, 0.96) | -2.14±2.42 | 0 (-5.10, 0) |
| BA+STS | 0.74±0.33 | 4 (0.47, 1.21) | 0.28±2.55 | 2 (-2.23, 3.33) | 0.90±1.08 | 3 (-0.63, 1.91) | -2.02±1.11 | 0 (-3.43, -0.73) |
| Red light | 1.26±1.63 | 3 (-0.87, 3.11) | 0.30±1.97 | 2 (-2.07, 2.66) | 1.32±0.86 | 4 (0.49, 2.15) | -2.61±1.26 | 0 (-4.41, -1.48) |
| PBZ | -4.62±3.04 | 0 (-7.59, -0.44) | -4.17±2.57 | 0 (-7.32, -1.02) | -0.61±1.66 | 2 (-2.17, 0.85) | -0.87±1.78 | 1 (-3.38, 0.79) |
| $KClO_3$ | -3.89±3.08 | 1 (-6.45, 0.28) | 0.10±1.71 | 2 (-1.97, 1.70) | -0.22±1.02 | 1 (-1.46, 1.03) | -0.51±1.46 | 1 (-1.62, 1.50) |
| **7 MAP** | | | | | | | | |
| Control | 3.13±0.57 | 4 (2.49, 3.87) | -4.72±3.35 | 0 (-7.33, 0) | 1.01±1.75 | 3 (-1.13, 3.15) | 0.00±0.00 | 0 (0) |
| BA+STS | 3.48±0.70 | 4 (2.87, 4.46) | 0.45±2.61 | 3 (-2.74, 3.65) | 0.82±2.22 | 3 (-1.71, 3.52) | -1.86±2.00 | 1 (-4.30, 0.58) |
| Red light | 1.88±2.43 | 3 (-1.40. 4.36) | -3.59±2.64 | 1 (-5.60. 0.14) | 0.56±1.24 | 3 (-0.55, 2.34) | 0.25±1.41 | 3 (-1.48, 1.98) |
| PBZ | -0.56±1.79 | 2 (-3.09, 0.85) | 1.36±1.64 | 3 (-0.90, 2.84) | 1.11±4.99 | 2 (-4.02, 6.70) | -2.23±1.45 | 0 (-4.00, -0.46) |
| $KClO_3$ | -1.53±3.19 | 2 (-5.34, 1.92) | 1.00±1.33 | 3 (-0.50, 2.75) | 1.15±1.92 | 2 (-0.36, 3.83) | -0.38±2.97 | 2 (-4.08, 2.84) |

The mean value of $log_2$ transformed gene expression level was shown with standard deviation. The range of gene expression levels in all replications was shown with the number of values higher than zero

**Gene expression level of *MeFT1* and *MeFT2* and correlation to cassava flowering traits:** For gene expression during the flowering period in Table 3, the gene expression level was performed by dividing the value of normalized gene expression by the value of R9 under the control group in the first replication of the same period, under the assumption that R9 might have gene expression level of *MeFT1* and *MeFT2* lower than those of HB80 which was the variety with high flowering potential. The gene expression level data were transformed in $log_2$ values. The negative values indicate the expression level which is lower than the expression level of R9 under the control group in the first replication. In Table 3, the values in each replication were shown in the range from the minimum value to the maximum value with the number of replications with positive values which indicated the values that were higher than that of R9 under the control group in the first replication.

At 5 MAP, the *MeFT1* expression levels of R9 under BA+STS condition in all four replications were above zero (averagely 2.89 and ranging from 1.52 to 4.09 in all four, replications). On the contrary, the number of flowering bunches of R9 at 5 MAP (Table 2) under BA+STS (totally 1 bunches) tended to be lower than that under control condition (totally 4 bunches). At 6 MAP, *MeFT1* expression levels of both HB80 and R9 under BA+STS (averagely 0.74 and 0.28, respectively) and red light (averagely 1.26 and 0.30, respectively) tended to be higher than those under control condition (averagely -0.09 and -0.01, respectively) which correspond with the pattern of flowering traits in both varieties (Table 1 and 2). At 7 MAP, there were many values of *MeFT1* expression level of HB80 under control (averagely 3.13 and having positive values in all 4 replications), BA+STS (averagely 3.48 and having positive values in all 4 replications) and red light (averagely 1.88 and having positive values in 3 replications) which were higher than zero which means the expression of *MeFT1* in HB80 tended to be higher than those of R9 under control that might contribute to flowering patterns at this period (Table 1). However, the above zero values of *MeFT1* expression level of R9 under BA+STS (averagely 0.45 and having positive values in 3 replications), PBZ (averagely 1.36 and having positive values in 3 replications) and $KClO_3$ (averagely 1.00 and having positive

Table 4: Correlation between the number of plants with flower bunches (Pl-B), the average number of bunches per plants (B), gene expression level of *MeFT1* and *MeFT2* in HB80 and R9 leaf sample collected in the same period from 5-7 MAP

| Correlation coefficient p-value | 5 MAP | 6 MAP | 7 MAP |
|---|---|---|---|
| Pl-B-B | 0.7477** | 0.7342** | 0.7679** |
| Pl-B-*MeFT1* | -0.0093$^{ns}$ | 0.3577* | 0.2239$^{ns}$ |
| Pl-B-*MeFT2* | -0.1018$^{ns}$ | 0.2695$^{ns}$ | 0.1060$^{ns}$ |
| B-*MeFT1* | 0.1275$^{ns}$ | 0.3866* | 0.3185* |
| B-*MeFT2* | 0.0275$^{ns}$ | 0.1300$^{ns}$ | 0.1764$^{ns}$ |
| *MeFT1*-*MeFT2* | 0.4092** | 0.1868$^{ns}$ | 0.2393$^{ns}$ |

**Significant correlation at p<0.01, *Significant correlation at p<0.05 and ns: No significant correlation

values in 3 replications) treatments did not cause flowering in R9 at this period (Table 2). Moreover, there were *MeFT1* expression levels above zero in some replications in both HB80 and R9 under PBZ and $KClO_3$ from 5-7 MAP except for the expression level under PBZ at 6 MAP (from -7.59 to -0.44 in HB80 and from -7.32 to -1.02 in R9 of all four replications) although there was no flowering in both varieties under PBZ and $KClO_3$ treatments at this first three-month period of flowering.

For *MeFT2* expression in Table 3, at 5 MAP, *MeFT2* expression level in R9 under $KClO_3$ (averagely 5.49) was significantly almost 32-fold (5-fold in $\log_2$ scale) higher than those of control (averagely 0.47) and BA+STS (averagely 0.81) treatment. However, at 5 MAP, R9 under $KClO_3$ did not have flowering yet in Table 2. There were positive values of relative *MeFT2* expression level in both HB80 and R9 under PBZ and $KClO_3$ at 5 MAP and 7 MAP except for the expression level under PBZ at 7 MAP (from -4.00 to -0.46 in R9 of all four replications) although there was no flowering in plants under PBZ and $KClO_3$ treatments at this period.

The *MeFT1* and *MeFT2* gene expression fluctuated throughout the experimental period. Several factors such as ambient temperature, light quality, sucrose concentration in the cell and vernalization may affect *FT* expression other than photoperiod and hormones[25,26]. Moreover, the cross-signal of environmental stress or conditions could affect differently on *FT* gene expression. Sucrose concentration in tissue was reported to be the direct inducer of *FT* gene expression, while, low nitrogen, water deficit condition under different photoperiods could have a different indirect effect on *FT* gene expression through the interaction on the upstream gene regulation[27]. Moreover, the high expression of both *MeFT1* and *MeFT2* during the period that plants did not have flowers, especially under PBZ and $KClO_3$ was the evidence to point out the possibility of other factors or genes for flowering induction or the delay effect of these genes on flowering.

In this experiment, there was no rainfall from December, 2019 through March 2020 which was the period from 6-9 MAP. Therefore, this might have an indirect effect on the fluctuation of *FT* gene expression pattern in this experiment and the level of *FT* mRNA might fluctuate throughout the day and night in the field condition as well. Even though there was a report that mRNA of *FT* genes which transferred to the apical meristem and lead to the flowering, this gene was regulated by *Hsp* promoter under heat induction to overexpression *FT* gene expression[17]. Other studies of *FT* gene expression in cassava confirmed its relationship with flowering induction were based on overexpression of the *FT* gene(s) using constitutive promoters, not by native promoters[18,19]. Moreover, grafting of high flowering cassava variety as the understock with the scion of low flowering genotypes could also induce earlier flowers flowering in erect varieties[28]. However, in this experiment, expressions of *FT* genes were not significantly triggered by any flowering-induction treatment which might be due to the reason that the number of transcripts of these genes was not high enough to initiate flowering as the over-expressed system or these florigens need other environmental factors such as low temperature to interact with.

From correlation analysis in Table 4, it shows that the number of plants with flower bunches (Pl-B) and the average number of bunches per plant (B) were significantly positively correlated (r = 0.7477, 0.7342 and 0.7679, respectively from 5-7 MAP). During 6-7 MAP. It also showed a significant correlation between *MeFT1* gene expression level and the number of flowering plants (r = 0.3577) and the average number of bunches per plant (r = 0.3866) at 6 MAP and between *MeFT1* gene expression level and the average number of bunches per plant (r = 0.3185) at 7 MAP. Interestingly, *MeFT1* and *MeFT2* expression levels were positively correlated (r = 0.4092) during 5 MAP when these gene expression levels did not correlate with flowering traits. However, during 6-7 MAP, when the *MeFT1* gene expression pattern correlated with flowering traits, the *MeFT2* gene expression pattern did not correlate with the *MeFT1* pattern or any flowering characteristics at all. Therefore, *MeFT1* might have the function on flowering in real-time which can be used as the indicator of flowering traits during the flourish period as Tokunaga *et al.*[21] reported on *MeFT1* and *MeFT2* expression pattern that *MeFT1* expression pattern was correlated with the flowering time of cassava, while *MeFT2* could express even in the period of no flowering.

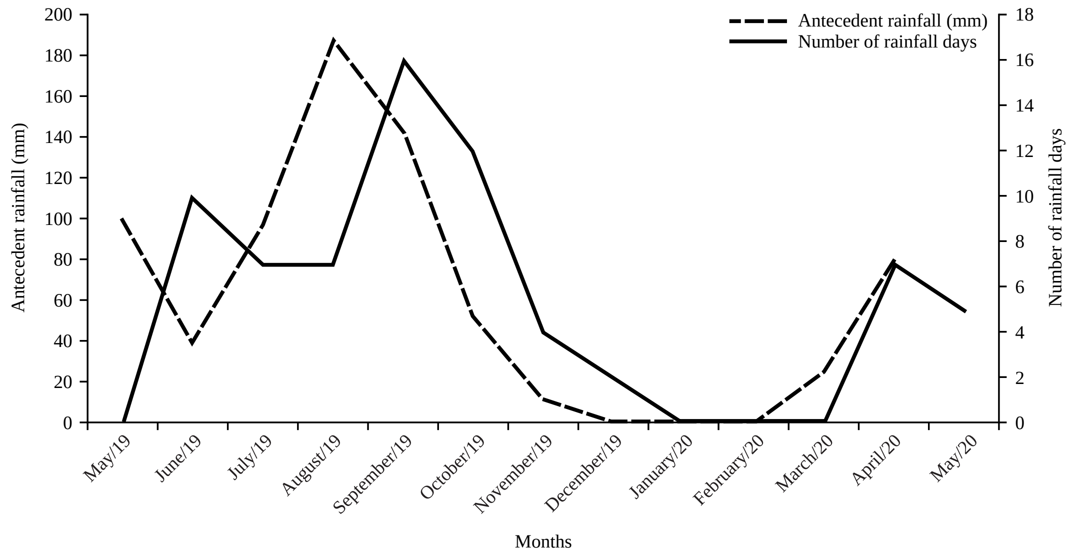


Fig. 2: Monthly rainfall (mm) accumulation and the number of rainfall days throughout the growing season at the experimental site, TDI, Huay Bong

Table 5: Correlation between the number of plants with flower bunches (Pl-B), the average number of bunches per plants (B), gene expression level of *MeFT1* and *MeFT2* in HB80 and R9 leaf sample collected in the different periods from 5-7 MAP

| Correlation coefficient p-value | *MeFT1* gene expression at 5 MAP | *MeFT2* gene expression at 5 MAP |
|---|---|---|
| Pl-B at 6 MAP | 0.0437$^{ns}$ | -0.1409$^{ns}$ |
| B at 6 MAP | -0.0900$^{ns}$ | 0.0346$^{ns}$ |
| Pl-B at 7 MAP | -0.0643$^{ns}$ | -0.0986$^{ns}$ |
| B at 7 MAP | -0.0409$^{ns}$ | 0.0461 |
| Correlation coefficient p-value | *MeFT1* gene expression at 6 MAP | *MeFT2* gene expression at 6 MAP |
| Pl-B at 7 MAP | 0.1922$^{ns}$ | 0.3682* |
| B at 7 MAP | 0.2057$^{ns}$ | 0.3322* |
| Pl-B at 8 MAP | -0.1756$^{ns}$ | 0.2851$^{ns}$ |
| B at 8 MAP | -0.1443$^{ns}$ | 0.2975$^{ns}$ |
| Correlation coefficient p-value | *MeFT1* gene expression at 7 MAP | *MeFT2* gene expression at 7 MAP |
| Pl-B at 8 MAP | 0.1138$^{ns}$ | 0.1901$^{ns}$ |
| B at 8 MAP | 0.1228$^{ns}$ | 0.1859$^{ns}$ |
| Pl-B at 9 MAP | 0.2378$^{ns}$ | 0.3618* |
| B at 9 MAP | 0.2486$^{ns}$ | 0.3760* |

*Significant correlation at $p<0.05$ and ns: No significant correlation

To investigate the delay effect of *MeFT1* and *MeFT2* function from leaves to flowering traits in 1 and 2 month-delay periods, the correlation between flowering traits (Pl-B and B) and *MeFT1* and *MeFT2* gene expression levels in leaves at 1 and 2 months before flowering data were analyzed in Table 5. There was no correlation between gene expression of both genes at 5 MAP to flowering traits at 6 and 7 MAP. Interestingly, there was a significant positive correlation between *MeFT2* expression level in leaves at 6 MAP to both flowering traits (r = 0.3682 in Pl-B and r = 0.3322 in B trait) at 7 MAP and there was a significant positive correlation between *MeFT2* expression level in leaves at 7 MAP to both flowering traits (r = 0.3618 in Pl-B and r = 0.3760 in B trait) at 9 MAP. However, there was no correlation between any gene expression and flowering traits at 8 MAP. Interestingly, at 8 MAP of cassava in this experiment, it was in February, 2020 which was the end of the dry spell period. In this location, rainfall began in May and ended in November. The accumulated rainfall in May was around 100 mm in one time of rainfall. Then, rainfall in June and July, decreased around 40 and 80 mm, respectively. Afterwards, the monthly accumulated rainfall rose again in August at 190 mm. Then, the rainfall started to decrease from September to November, at 150, 50 and 10 mm, respectively. From December to February, there was least than 1 mm of rainfall and then the rainfall returned in March around 20 mm in Fig. 2 in this location. Therefore, the low soil moisture might cause plants severe water deficit conditions which affected flowering availability as mentioned in Cho *et al*.[27]. The significant positive correlation between *MeFT2* gene expression and flowering traits started at 6 MAP when most cassava varieties had the high number of flowering bunches in this experiment, but not at 5 MAP when cassava flowering was started. Therefore, these data suggest that there are more environmental interacting factors or genes involved in cassava flowering. However, according to the result of this experiment, *MeFT2* has the potential to be used as an early indicator for cassava flowering in the following month if there is no factor of drought occurring in the field which will be beneficial for crossing plan in the breeding program.

## CONCLUSION

Early flowering varieties, HB80, responded well for flowering under BA+STS induction. Branching induction was not related to flowering induction in this experiment. The expression of *MeFT* genes under different flowering induction conditions in this experiment fluctuated which might be due to the warm temperature factor suggesting for the further experiment at the lower temperature location. The *MeFT1* transcript level tends to have an immediate effect on cassava flowering, whereas, *MeFT2* gene expression showed the delay effect on flowering in the following month. Complicating matters further is the genetic and the potential genetic and environmental interactions that may be expected to influence flowering in cassava.

## ACKNOWLEDGMENT

This study fund was granted by the research fund from the National Science and Technology Development Agency (NSTDA) with the support of an experimental facility by Tapioca Development Institute (TDI), Huay Bong. The first author was granted from Kasetsart University Graduate School Scholarship 2019.

## SIGNIFICANCE STATEMENT

This study discovered the association of the *MeFT1* expression pattern with cassava flowering potential at the same period and the correlation of the *MeFT2* expression pattern with cassava flowering trend in the following month if there was no drought stress occurred. This finding can be a benefit for cassava breeders to use *MeFT2* expression data to precisely manage for crossing plan in the following month. This study will help the researchers to uncover the critical areas of cassava flowering induction that have never been explored before.

## REFERENCES

1. Ceballos, H., C. Rojanaridpiched, C. Phumichai, L.A. Becerra, P. Kittipadakul, C. Iglesias and V.E. Gracen, 2020. Excellence in cassava breeding: Perspectives for the future. Crop Breed Genet. Genom., Vol. 2. 10.20900/cbgg20200008.
2. Malik, A.I., P. Kongsil, V.A. Nguy n, W. Ou and Sholihin *et al*., 2020. Cassava breeding and agronomy in Asia: 50 years of history and future directions. Breed. Sci. 70: 145-166.
3. Carvalho, R.D. and M. Guerra, 2002. Cytogenetics of *Manihot esculenta* Crantz (cassava) and eight related species. Hereditas, 136: 159-168.
4. Soto, J.C., J.F. Ortiz, L. Perlaza-Jiménez, A.X. Vásquez and L.A.B. Lopez-Lavalle *et al*., 2015. A genetic map of cassava (*Manihot esculenta* Crantz) with integrated physical mapping of immunity-related genes. BMC Genom., Vol. 16. 10.1186/s12864-015-1397-4.
5. Rojas, M.C., J.C. Pérez, H. Ceballos, D. Baena, N. Morante and F. Calle, 2009. Analysis of inbreeding depression in eight $S_1$ Cassava families. Crop Sci., 49: 543-548.
6. Adeyemo, O.S., P.T. Hyde and T.L. Setter, 2019. Identification of FT family genes that respond to photoperiod, temperature and genotype in relation to flowering in cassava (*Manihot esculenta*, Crantz). Plant Reprod., 32: 181-191.
7. Perera, P.I.P., M. Quintero, B. Dedicova, J.D.J.S. Kularatne and H. Ceballos, 2013. Comparative morphology, biology and histology of reproductive development in three lines of *Manihot esculenta* Crantz (Euphorbiaceae: Crotonoideae). AoB Plants, Vol. 5. 10.1093/aobpla/pls046.
8. Pineda, M., N. Morante, S. Salazar, J. Cuásquer, P.T. Hyde, T.L. Setter and H. Ceballos, 2020. Induction of earlier flowering in cassava through extended photoperiod. Agronomy, Vol. 10. 10.3390/agronomy10091273.
9. Pineda, M., B. Yu, Y. Tian, N. Morante and S. Salazar *et al*., 2020. Effect of pruning young branches on fruit and seed set in cassava. Front. Plant Sci., Vol. 11. 10.3389/fpls.2020.01107.
10. Oluwasanya, D., O. Esan, P.T. Hyde, P. Kulakow and T.L. Setter, 2021. Flower development in cassava is feminized by cytokinin, while proliferation is stimulated by anti-ethylene and pruning: Transcriptome responses. Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.666266.
11. Srilatha, V., Y.T.N. Reddy, K.K. Upreti, R. Venugopalan and H.L. Jayaram, 2016. Responses of pruning and paclobutrazol in mango (*Mangifera indica* L.): Changes in tree vigour, flowering and phenols. J. Appl. Hort., 18: 148-153.
12. Desta, B. and G. Amare, 2021. Paclobutrazol as a plant growth regulator. Chem. Bio. Technol. Agric., Vol. 8. 10.1186/s40538-020-00199-z.
13. Kumar, A., C.P. Singh and L.D. Bist, 2019. Effect of paclobutrazol (PP333) on growth, fruit quality and storage potential of mango Cvs. Dashehari, Langra, Chausa and Fazri. Eur. J. Agric. For. Res., 7: 23-37.

14. Kumar, A., S. Ram and C.P. Singh, 2020. Application methods of paclobutrazol in mango Cvs & its residual effect in leaf, fruit and orchard soil. J. Res. Agric. For., 7: 24-39.
15. Yuliadi, E. and Ardian, 2016. Flower Induction of Cassava (*Manihot esculenta* crantz) Through the Application of Paclobutrazol and $KNO_3$. The USR International Seminar on Food Security (UISFS), August 23-24, 2016, Bander Lampung, Indonesia, 149-158.
16. Unpanrim, S., 2002. Effect of potassium chlorate, potassium nitrate and thiourea on flowering and carbohydrate content of Longan. Kasetsart University, Bachelor of Science, Bangkok (Thailand). http://www.lib.kps.ku.ac.th/SpecialProject/Science/2545/Bs/SuthathipUr/SuthathipUrAll.pdf
17. Huang, T., H. Bo¨hlenius, S. Eriksson, F. Parcy and O. Nilsson, 2005. The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. Science, 309: 1694-1696.
18. Adeyemo, O.S., P. Chavarriaga, J. Tohme, M. Fregene, S.J. Davis and T.L. Setter, 2017. Overexpression of arabidopsis *Flowering locus T* (FT) gene improves floral development in cassava (*Manihot esculenta*, Crantz). PLoS ONE, Vol. 12. 10.1371/journal.pone.0181460.
19. Bull, S., A. Alder, C. Barsan, M. Kohler, L. Hennig, W. Gruissem and H. Vanderschuren, 2017. *Flowering locus T* triggers early and fertile flowering in glasshouse cassava (*Manihot esculenta* Crantz). Plants, Vol. 6. 10.3390/plants6020022.
20. Kumar, G.N.M., S. Iyer and N.R. Knowles, 2007. Extraction of RNA from fresh, frozen, and lyophilized tuber and root tissues. J. Agric. Food Chem., 55: 1674-1678.
21. Tokunaga, H., D.T.N. Quynh, N.H. Anh, P.T. Nhan and A. Matsui *et al*., 2020. Field transcriptome analysis reveals a molecular mechanism for cassava-flowering in a mountainous environment in Southeast Asia. Plant Mol. Biol., 10.1007/s11103-020-01057-0.
22. Souza, L.S., A.A.C. Alves and E.J. de Oliveira, 2020. Phenological diversity of flowering and fruiting in cassava germplasm. Sci. Hort., Vol. 265. 10.1016/j.scienta.2020.109253.
23. Ibrahim, Y., Y. Baguma, W. Abincha, P. Gibson, R. Edema and J. Bisikwa, 2020. Flowering problems and their possible solution in cassava breeding. J. Sci. Agric., 4: 83-89.
24. Oluwasanya, D.N., A. Gisel, L. Stavolone and T.L. Setter, 2021. Environmental responsiveness of flowering time in cassava genotypes and associated transcriptome changes. PLoS ONE, Vol. 16. 10.1371/journal.pone.0253555.
25. Saedler, H., A. Becker, K.U. Winter, C. Kirchner and G. Theissen, 2001. MADS-box genes are involved in floral development and evolution. Acta Biochim. Pol., 48: 351-358.
26. Corbesier, L. and G. Coupland, 2006. The quest for florigen: A review of recent progress. J. Exp. Bot., 57: 3395-3403.
27. Cho, L.H., J. Yoon and G. An, 2017. The control of flowering time by environmental factors. Plant J., 90: 708-719.
28. Ceballos, H., J.J. Jaramillo, S. Salazar, L.M. Pineda, F. Calle and T. Setter, 2017. Induction of flowering in cassava through grafting. J. Plant Breed. Crop Sci., 9: 19-29.