Science & Technology Development Journal 2023, 26(3):2943-2949

Open Access Full Text Article

Natural Sciences: Research Article

Study on flowering in rice plants (*Oryza sativa* cv. OM5451)

Linh Minh Hong Tran*, Huong Thanh Tran*, Viet Trang Bui

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ABSTRACT

Introduction: The consumption of rice is rising in response to worldwide population development. The purpose of this study was to determine the stages of plant development, specifically the transition stage from panicle initiation to the flowering stage, which determines the number of spikelets per branch. In addition, this research studied the changes in physiology and morphology during the flowering development of rice in a garden. **Methods**: Plant height, number of leaves, number of tillers, and flowering time were all measured. Plant growth regulators such as cytokinin, auxin, gibberellic acid (GA), and acid abscisic (ABA) in the apical shoot at different stages of flower development were analyzed. **Results**: The activities of indole acetic acid (IAA), zeatin, and gibberellins in the apical shoot of the stem elongation stage are higher than those in plants of the panicle initiation stage. At the flowering stage, the rates of photosynthesis and respiration of the leaves at positions 1, 2, and 3 were higher than those of the other leaves. In addition, photosynthetic pigments at leaf positions 2, 3, and 4 were higher than those at positions 1 and 3. **Conclusion**: The results show that the relationship between changes in morphological, physiological, and biochemical indicators during the rice growth stages was discussed.

Key words: flower development, flowering time, Oryza sativa, plant growth regulators

Faculty of Biology and Biotechnology, VNUHCM- University of Science, Vietnam

Correspondence

Linh Minh Hong Tran, Faculty of Biology and Biotechnology, VNUHCM- University of Science, Vietnam

Email: tranminhhonglinh@gmail.com

Correspondence

Huong Thanh Tran, Faculty of Biology and Biotechnology, VNUHCM- University of Science, Vietnam

Email: trthuong@hcmus.edu.vn

History

- Received: 2023-05-12
- Accepted: 2023-09-05
- Published Online: 2023-09-30

DOI :

https://doi.org/10.32508/stdj.v26i3.4088

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INTRODUCTION

Rice (*Oryza sativa* L.) provides a significant source of nutrients and plays an important role in global food security for more than 50% of the populace, particularly in Asia, and its production is urgently needed. As one of the most significant harvests in the world, rice is a staple food for nearly 2.7 billion people, and production levels must grow by 50% by 2050 to meet rising demand [1]. In addition, rice is cultivated in a variety of climates. With approximately 162 million hectares of agricultural land and a yield of 755 million tonnes, it is planted in over 110 countries worldwide, with an average yield of 4.66 metric tons per ha [1,2].

In important crops such as rice, inflorescence development directly impacts the number and size of seeds and is a major determinant in production. Meristematic mechanisms that govern branching patterns and flower location establish the basis for inflorescence development early in reproductive development [3]. Plant hormones are essential for regulating all aspects of the growth, development, and metabolism of plants. The development and structure of inflorescences are significantly influenced by cytokinin, a fundamental regulator of meristem size and activity [3,4]. The level to which cytokinins are expressed at various early panicle developmental stages suggests that their expression could have different impacts on inflorescence morphology [2]. In rice, as in most plants, genes involved in cytokinin activity control the development of an important crop's inflorescence, with implications for similar processes in other monocots [5,6]. Auxin is a typical phytohormone that is essential for plant morphogenesis and a variety of physiological activities, including cell differentiation, cell elongation, flowering development, and germination [7]. Auxin signaling and distribution in rice tissues have not been considerably studied, in contrast to the model eudicot plant *Arabidopsis*, due to a lack of adequate auxin response reporters [3]. GAs are vital and effective regulators of plant growth and developmental activities, such as promoting the germination of seeds, lengthening stems, and controlling bolting and blooming. GAs regulate stem elongation by short-day photoperiod signals. In rice grown under noninductive long days, GA may also be necessary for floral changes. Additional molecular investigation is necessary to determine the involvement of GAs in rice flower initiation regulation. In *A. thaliana*, GA signals are important for the "competence to flower" [6]. Other reviews frequently highlighted the possibility that GAs could be florigenic [8]. Recent research has revealed that ABA signals under stress can impact the flower transition to flowering earlier than plants under normal conditions [9]. However, it is presently unclear exactly how ABA affects floral transitions.

The objective of this research was to study flower development in rice. This study improves our under-

Cite this article : Tran L M H, Tran H T, Bui V T. **Study on flowering in rice plants (*Oryza sativa* cv. OM5451)**. *Sci. Tech. Dev. J.* 2023; 26(3):2943-2949.

standing of how phytohormones cause flowering and provides a scientific basis for rice production.

MATERIALS — METHODS

Plant materials and cultivation methods

The seeds of the OM5451 variety were provided by the Cuu Long Delta Rice Research Institute in Vietnam. The seeds were soaked in water at approximately 50°C for 15 minutes. Afterwards, these seeds were rinsed with water and soaked at 35°C for an additional 24 hours. The seeds were then kept in the dark for 24 hours to allow them to sprout until the roots reached a length of approximately 2 mm. The seedlings were then sown in Styrofoam boxes 40 centimeters (cm) in height, 60 centimeters in length, and 30 centimeters in width. In each box, 24 plants were planted in Tribat soil with a pH range of 6.0 to 6.5 (Tribat soil contains 15% humus, 25% organic matter, 1% total N, 0.73% total K2O%, and 0.3% total P_2O_5). Rice plants were fertilized according to the formula 100 kgN/ha - 40 kgP_2O_5/ha - 30 kgK_2O/ha, with the amount of fertilizer depending on the growth stage. The distance between plants was 10 cm. The experiment was placed in a garden with a temperature varying between 30 to 35°C during the day and 24 to 26°C at night, 10/14 hour photoperiods at 110000 lux (at 12 noon) and relative humidity of 65 - 75%. During the vegetative stage, plants in all treatments were kept flooded to maintain a layer of 5 - 6 cm depth of water. At the beginning of the reproductive stages, plants were kept at a depth of 2-3 cm of water, and all boxes were drained 15 days before harvest.

The height of the plants, number of leaves per plant, number of tillers, and length of the leaves on the main stem were measured at different developmental stages. The area of the leaf at different positions (1-5 from the apical meristem) was measured and determined using a method adapted by LeafByte (Getman-Pickering). To prevent border effects, the border rows were not collected from the experimental plots. In addition, the number of panicles and leaves per plant was also recorded.

The treatments were designed with a randomized complete block for four replications, and each replicate included three boxes. The growth of the rice was observed. The flowering time, length and area of the flag leaf, and plant height were determined on day sixty-one (the flowering time of the control plant).

Observation of morphological changes

The apical shoots of rice plants were collected at the vegetative and flowering stages and then placed in FAA solution (acetic acid:formalin:95% ethanol:H_2O at a ratio of 5:10:50:35 by volume). After 17 hours, the FAA and any remaining water in the sample were removed by a series of ethanol and butanol[10]. After being dehydrated, the shoot was placed in paraffin solution at 56°C. Then, the shoot was longitudinally cut into 9 μm slices by using a manual rotary microtome (Rotary microtome, Microm HM304E). The slices were then submerged in solutions of methylcyclohexane, ethanol, and water to remove the paraffin. Finally, the shoot was stained with double-staining carmine aluminum and iodine green and observed under an optical microscope (ACCU-Scope, USA).

Extraction, isolation, and quantitative analysis of phytohormones

Plant hormones such as cytokinin, auxin, gibberellin, and ABA were extracted from apical shoots at various developmental stages by using methanol, diethyl ether, and pH alterations. A thin-layer chromatogram (60 F254, 105554, Merck) on silica gel was used to isolate the plant hormones at 30°C with the solvent mixture isopropanol: ammonium hydroxide: H_2O (10:1:1 v/v). Under ultraviolet light, hormones in plants were found. By using a bioassay technique, the amount of plant hormones was determined. The activities of zeatin, gibberellin, auxin, and abscisic acid were assessed in cucumber cotyledons, lettuce hypocotyl, and rice coleoptile sections, respectively[11,12]. The results were measured with three replicates.

Analysis of physiological and biochemical changes in plants

Determination of chlorophyll

Leaves were taken from the top to the bottom of the plant at the heading stage for chlorophyll quantification. Leaves (1.0 g) were crushed with 10 ml of ethanol (95%), and the homogenates were centrifuged at 10000 × g for 5 min to obtain the supernatant. The optical density of the extract was measured at 470 and 648 nm with a UV−VIS spectrometer (Shanghai Metash Instrument Co., Ltd, China). The chlorophyll a and chlorophyll b contents were obtained according to the formula given by Lichtenthaler[13].

Chlorophyll a = 13.36 A_{664}−5.19 A_{648}

Chlorophyll b = 27.34 A_{648}−8.12 A_{664}

Determination of respiration and photosynthesis intensity

The intensity of respiration and photosynthesis ($\mu mO_2/cm^2$/hour) of leaves at different positions was

determined by an oxygen electrode meter (LeafLab2, Hansetech, UK). The respiration intensity of leaves was determined in the dark by an oxygen electrode based on the decrease in oxygen in the measuring chamber. The intensity of photosynthesis of leaves was determined by an oxygen electrode based on the increase in oxygen in the measuring chamber under 10000 lux light at a temperature of 27°C. The result is the average value of three measurements.

Statistical analysis

A randomized block design (RBD) with replications was used for all treatments. Using one-way analysis of variance, data were analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) to determine the least significant difference at P 0.05. The results of the experiment are presented as the mean ± standard deviation (SD).

RESULTS

The development of rice grown in the garden

The vegetative and reproductive phases are subdivided into different stages. There are three stages in the vegetative phase: (1) emergence, (2) tillering, and (3) internode elongation. The reproductive phase is divided into four stages: (1) panicle initiation, (2) booting, (3) heading and fertilization, and (4) maturity (Figure 1, Table 1). Plant height gradually increased throughout development, reaching its highest value at the heading stage (94.25 cm). The plant begins tillering on day twenty-two. Then, the number of tillers gradually increased and reached the highest number (4.4 tillers/plant). In addition, the number of leaves per plant appears to increase over time, reaching its peak at the heading stage (11.2 leaves per plant).

Morphological changes in rice flowering

The shoot apical meristem (SAM) before panicle production is referred to as stage 0 (ST0), with developing leaf primordia. The early stages of rice panicle growth were separated into three stages (1–3): (1), inflorescence meristem (IM) initiation with the elongation meristem priority branches; (2), secondary branch (SB) initiation; and (3), spikelet meristem (SM) establishment (Figure 2). The spikelet meristems developed into floret meristems (FM) once the hood-shaped lemma and palea primordia were formed (Figure 3). The FM generated lodicule primordia, which were concealed within the enclosing lemma and palea, stamen primordia, and a carpel primordium consecutively (Figure 3).

Changes in plant growth regulators during shoot development

The activity of zeatin, IAA, and GA_3 in the apical shoots increased from the tillering to the elongation stage and then remained unchanged at the panicle initiation stage. Meanwhile, ABA activity showed no significant changes in the shoots (Table 2).

Physiological and biochemical changes in plants

Of all the leaves on the plant from top to bottom, the leaves with the largest area are leaves 2 and 3 (48.50 and 49.61 cm^2, respectively), and the remaining leaves have a leaf area with no significant differences. There was a significant difference in the rate of leaf photosynthesis and respiration. The photosynthesis and respiration of leaves 1, 2, and 3 (top leaves) were higher than those of other leaves (Table 5). Photosynthetic pigments such as chlorophyll a and b are high in leaf positions 2, 3 and 4. Leaves at position 5 had the lowest content of photosynthetic pigments.

DISCUSSION

During rice plant development, the height of plants, the number of leaves, and the number of tillers increased significantly. During the tillering stage, the height increases rapidly to assist the plant in extending the stem, and the number of leaves is continually increased to aid in photosynthesis. In the reproductive stage, the flag leaf is the last leaf to be formed. In addition, at the heading stage, the intensity of photosynthesis and respiration of leaf numbers 1, 2, and 3 significantly increased to provide energy for the plant and precursor compounds for synthesis, especially starch synthesis during the grain filling stage. It has been demonstrated that chlorophyll b and chlorophyll a are light-absorbing pigments that accumulate and transport more light energy, increasing their photosynthetic efficiency. Increased sunlight during rice flowering improves the metabolism of carbon and increases the synthesis of starch in the endosperm [14].

In the early stages of reproduction (panicle initiation stage), meristematic processes that determine flowering time and flower positioning set the foundation for inflorescence architecture. The earliest sign of panicle development in rice is the shoot apical meristem (SAM) of longitudinal extension. The IM initiates primary branch meristems (pBMs) in rice, which subsequently initiate secondary branch meristems (sBMs) and then establish spikelet meristems (SMs). The spikelet meristem (SM), which differentiates floral organs, will develop into a floral meristem

Table 1: Development and growth indicators in each stage of garden development

Growth stages	Day number	Plant height (cm)	Tiller number/plant	Number of leaves/plant
Emergence	-	-	-	-
Tillering	21.77 ± 0.29^{f}	-	2.48 ± 0.13^{c}	4.54 ± 0.20^{d}
Internode elongation	30.29 ± 0.59^{e}	1.25 ± 0.10^{e}	3.25 ± 0.12^{b}	6.52 ± 0.16^{c}
Panicle initiation	35.52 ± 0.34^{d}	22.18 ± 0.68^{d}	4.31 ± 0.21^{a}	8.69 ± 0.13^{b}
Booting	45.15 ± 0.53^{c}	30.48 ± 0.72^{c}	4.27 ± 0.18^{a}	11.04 ± 0.11^{a}
Heading and fertilization	62.04 ± 0.20^{b}	53.76 ± 0.80^{b}	4.35 ± 0.08^{a}	11.02 ± 0.30^{a}
Maturity	84.23 ± 0.18^{a}	93.25 ± 1.06^{a}	4.42 ± 0.07^{a}	11.19 ± 0.20^{a}

Values with different letters in a column are significantly different according to Duncan's test ($p = 0.05$)
(–) *There is no interaction between the factors*

Figure 1: **Rice plants were grown in the garden at different stages**. (**A**) Emergence stage on the first day; (**B**) Tillering stage on the twenty-two day; (**C**) Internode elongation stage on the twenty-ninth day; (**D**) Panicle initiation stage on the third-six day; (**E**) Heading and fertilization on the sixty-one day; (**F**) Maturity stage at the eighty-one day. Scale bar = 1 cm

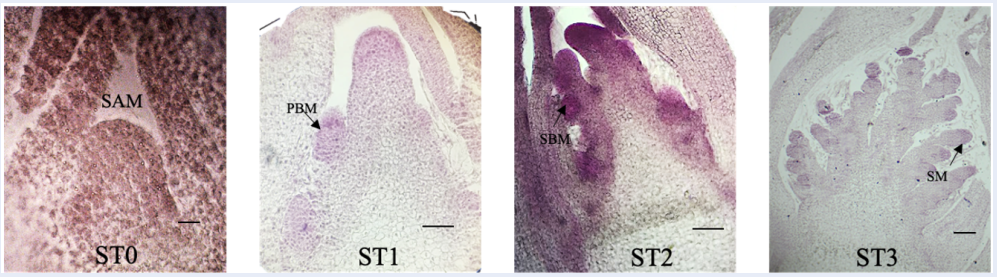

Figure 2: **Early stages of rice panicle formation**. Stage 0 (ST0), SAM just before the change from vegetative to reproductive development; Stage 1 (ST1), the stage of primary branch meristems (PBM); Stage 2 (ST2), secondary branch meristems (SBM); Stage 3 (ST3), the initiation of the floral organ with SM. Scale bar = 50 μm.

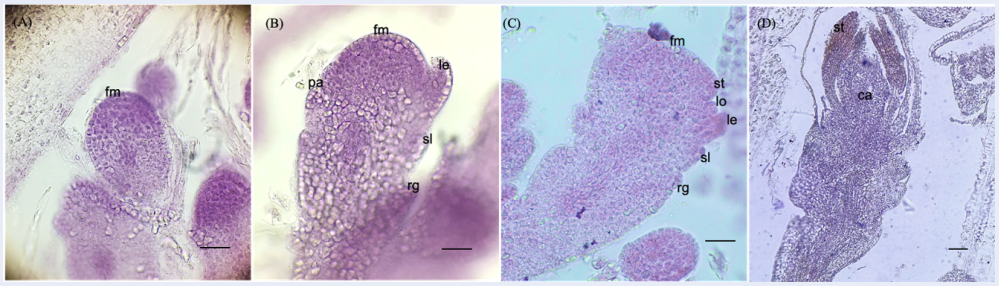

Figure 3: **Spikelet meristems (SMs) at various developmental stages**. (**A**) Early floret meristem (FM), (**B**) Floret within the formed stamens, lemma/palea organ primordia. (**C**) The lodicule and stamens in the near mature lemma and palea. (**D**) In the central early carpel primordia. (rg, rudimentary glume; sl, sterile lemma (glume); le, lemma; pa, palea; lo, lodicule; st, stamen; ca, carpel). Scale bar = 50 μm.

Table 2: Changes in plant growth regulators in apical shoots from the vegetative stage to the panicle initiation stage

Growth stages	Content of plant growth regulators (mg L-1)			
	Zeatin	GA_3	IAA	ABA
Tillering (23 days)	0.53 ± 0.17^b	0.80 ± 0.15^b	0.50 ± 0.17^b	0.17 ± 0.07^a
Elongation (30 days)	1.39 ± 0.12^a	1.37 ± 0.25^a	1.12 ± 0.30^a	0.18 ± 0.05^a
Panicle initiation (36 days)	1.83 ± 0.56^a	1.63 ± 0.37^a	1.30 ± 0.28^a	0.16 ± 0.05^a

Values with different letters in a column are significantly different according to Duncan's test (p = 0.05)

Table 3: Changes in leaf area, chlorophyll content, photosynthesis, and respiration intensity according to leaf position at the heading stage

Leaf position	Leaf area (cm^2)	Content ($mg.g^{-1}$)		Intensity (μmolO2/cm2/hour)	
		Chlorophyll a	Chlorophyll b	Photosynthesis	Respiration
1	42.66 ± 1.94^b	3.07 ± 0.05^b	0.95 ± 0.05^c	22.32 ± 0.71^a	12.08 ± 0.25^a
2	48.50 ± 1.09^a	4.77 ± 0.03^a	1.15 ± 0.08^b	22.07 ± 1.14^a	11.84 ± 0.36^{ab}
3	49.61 ± 1.84^a	4.77 ± 0.05^a	1.09 ± 0.07^b	20.60 ± 0.68^{ab}	11.89 ± 0.62^{ab}
4	50.09 ± 1.77^a	3.13 ± 0.04^b	1.29 ± 0.07^a	18.99 ± 1.77^b	10.98 ± 0.87^b
5	39.81 ± 1.80^c	2.97 ± 0.03^c	0.87 ± 0.02^c	18.05 ± 1.11^c	8.70 ± 0.44^c

Values with different letters in a column are significantly different according to Duncan's test (p = 0.05)

(Figure 3a). Glumes, also known as bract-like, cover all spikelets [15]. One spikelet in rice has a fertile floret and a combination of lemma and palea (also known as 'empty glumes'), which are subtended by a pair of reduced rudimentary glumes [16]. The inner floral organs, the lodicule, stamen, and pistil, are covered in the grass-specific perianth organs known as the palea and lemma [17]. These organs are formed by the FM (Figure 3B, C, and D). The spikelet, a structure resembling the dicot flower, is the defining characteristic of the recurrent reproductive structure of all grass inflorescences [14].

In rice, PGRs are essential for the coordination of several behavioral and growth mechanisms, which determine the rate, kind, and path of plant development [18,19]. In rice, floral meristem changes are followed by stem elongation, as shown in Table 1. GA-dependent flowering was found to be localized in two main areas: the leaves and the shoot apex [6,20]. Auxin and cytokinin are the two main phytohormones that directly regulate the differentiation and proliferation of cells via cell growth and division regulation. However, the mechanisms by which these plant hormones contribute to flowering transition are

mainly unknown[21]. According to Table 1, auxin and cytokinin were both high during flower development to stimulate the organogenesis of flowers. Many roles for IAA during grain filling have been indicated by hormones in growing endosperm[7]. Studies from other cereals indicate that cytokinin is important in a flower's early growth and development. For a variety of cereals, such as rice (*Oryza sativa* L.), cytokinin impacts shoot growth, inflorescence morphology, flower organ, fertilization, and endosperm cell number[19].

CONCLUSIONS

In the present study, the plant height, number of leaves, and number of tillers increased during the vegetative stage of rice. During the heading stage, the plant develops plant height, photosynthesis, and respiration activity of the three leaves on the top of the plant. The activity of auxin, cytokinin, and GA_3 increases in the stem elongation stage to cell division and growth.

ACKNOWLEDGMENT

This research is funded by Vietnam National University, Ho Chi Minh City (VNU-HCM) under grant number T2021-15.

ABBREVIATIONS

BA: 6-Benzyl aminopurine
GA_3: Gibberellic acid
IAA: Indole acetic acid
ABA: Acid abscisic
PGRs: Plant growth regulators

AUTHOR CONTRIBUTION

Linh MHT contributed directly to conducting experiments, collecting and processing data, and writing manuscripts. Huong TT and Viet BT made important contributions to the analysis and interpretation of the results in the manuscript.

COMPETING INTERESTS

The authors declare no competing financial interests.

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