Study on the formation and development of aromatic rice spikelets

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Abstract:

In order to detect some of the determinants of rice flavor, an experiment was carried out that studied panicle primordia at the initial panicles of three aromatic varieties: Nang Thom Cho Dao mutation (NTCDm), Thom Bay Nui (TBN), and Jasmine-85, and IR28 was used as the control variety (non-aroma). The samples were dyed from the primordium stage to the ripe-pollen stage. Results showed that there were two key differences between the aromatic rices and the control. The first point of difference was at the primordium stage, and the second was after, as a consideration of the number of bivalents (pairs of homologous chromosomes). As for the three aromatic rice varieties, there appeared a lobe division at the branch primordium; at the diplotene stage of meiosis, and seven to eight stained bivalents appeared while the control had no lobe division and the number of stained bivalents achieved 11 to 12.

Keywords: ***meiosis, primordia stage, rabl configuration, rice flavor.***

Classification number: ***3.1***

Introduction

In recent years, the production of some famous aromatic rice varieties have been increasing, including Jasmine 85 and NTCDm. Their yields have been ranging from three to five tonnes/ha [1]. In addition to this, the quality of aromatic rice varieties have been distinguished as cooking rice and rice grain that is shiny, fragrant [2], delicious and is many consumers first choice for daily meals; while Vietnam's fragrant rice is not stable and the smell does not keep long.

Twelve consecutive photoinductive cycles have established the full shape of the panicle along with the differentiation of the lodicules, anthers and the pistil primordium in the individual spikelets borne at the apical region (Misra and Khan, 1969). In the P4 leaf primordium, strong OSHB3 expression was evident in the adaxial cells of the ligule primordium (Itoh, *et al.,*2008). Spikelet lengths varied significantly among the genotypes. Minimum spikelet length was recorded in Kalijira (White type), while the maximum length was observed in Kaloshailla (P.S. Saha, *et al.*, 2015).

Research of the formation and development of aromatic rice flowers rarely is published. Rice breeding on aromatic rices is very difficult for rice breeder. Thus, finding an indicator to study aroma was important in order to select new aromatic rices. The objective of the study was to detect the formation and development of aromatic rice spikelets at the initial primordium stage.

Material and method

Material

Seeds: seeds of three aromatic rices: NTCDm, Jasmine 85, and TBN; and the non-aromatic rice, IR28, were studied (Table 1). The seeds were provided by the Department of Genetics and Plant Breeding, at the College of Agriculture and Applied Biology, at Can Tho University.

Methods

Sample soil preparation: seeds were soaked in water for 24 hours, then allowed to germinate for 48 hours. When seedlings grew to 2-3 cm, they were transplanted directly into a ceramic pot (30x26x16 cm). Soil in the pot was prepared as follows: 120 g of compost (manure), 0.72 g P_2O_5, 0.36 g K_2O (100N-60P_2O_5-60K_2O), and water.

Layout: the experiment was arranged as a randomized complete block, four treatments with three replications.

Methods of staining samples

Prepare materials: the meiosis was

Table 1. Some agronomical characteristics of rice varieties used in this experiment.

Characteristic	NTCDm	Jasmine 85	TBN	IR28
Growth duration (days)	110-115	105-110	120-160	90-95
Plant height (cm)	115-145	110-115	120-150	95-100
Yield (t/ha)	3.5	4-6	3-4	7-8
Amylose content (%)	21.26	18-22	14.8	16.03
Chalkiness (%)	0	0	0	0.3
Grain length (mm)	6.3	6.9	7.8-8.0	6.9
Weight of 1,000 grains (g)	27	26	27.2-27.8	27.15

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Table 2. Developmental stages and morphological characteristics[a].

Developmental stages	Morphological characteristics		
	Leaf index (%)	*Exertion of n^{th} leaf counted from the top*	*Panicle length (mm)*
1. Necknode differentiation stage	76-78	4th leaf	
2. Branch differentiation stage	80-86	3rd leaf	
3. Spikelet differentiation stage	87-92	2nd leaf	1-15
4. Pollen mother cell differentiation stage	95	Flag-leaf	15-50
5. Reduction division stage of pollen mother cell	97		50-200
6. Extine formation stage	100		Full length
7. Ripe pollen stage	100		Full length

[a]Modified from Matsushima (1970)

observed when the flower buds were very young (around 50 days after sowing), and panicle length was about 7-8 cm. After cutting the buds from the plant, they were fixed within Carnoy's solution [3].

Staining: the samples were stained with Aceto-Carmine, or the chromosome or cell nuclei would become red, while the rest was pale pink.

Working method: stained specimens were placed in microscope slides covered with lamella, then heated lightly over an alcohol lamp, and pressed lightly by thumb. Chromosomes (if present in the cells) were clearly visible in the microscope.

Methods to identify the stages of spikelet formation and development: to identify the stages of spikelet formation and development, the method of [4] was applied (Table 2).

Results and discussion

Morphological variation of rice spikelet primordium of IR28 and NTCDm

The initiation of the panicle primordium of IR28 began about 25 days before heading, and the remaining experiment lasted for about 30 days. The method was suitable for the time, until the fourth leaf from the top began to elongate. The major elements of the panicle were the base, axis, primary, and secondary branches, pedicels, rudimentary glumes, and spikelets (Fig. 1). The spikelet was borne on the pedicel, and a short stalk was developed as an extension of the panicle axis at the primary or secondary branch. There were two short rudimentary glumes at the upper end of the pedicel. A pair of sterile lemmas and the rachilla were located between the rudimentary glumes and the spikelet. The flower was enclosed in the lemma and palea. The flower consisted of the pistil, stamens, and lodicules. The components of the pistil were the stigmas, styles, and ovary. The stigma had plumose, on to which pollen grains were stored for germination.

There were six well-developed stamens composed of anther and filament. Two small, oval, thick, and fleshy bodies, called the lodicules, were situated at the base of the ovary. The lodicules became distended with water and assisted in separating the lemma and palea when it flowered, this

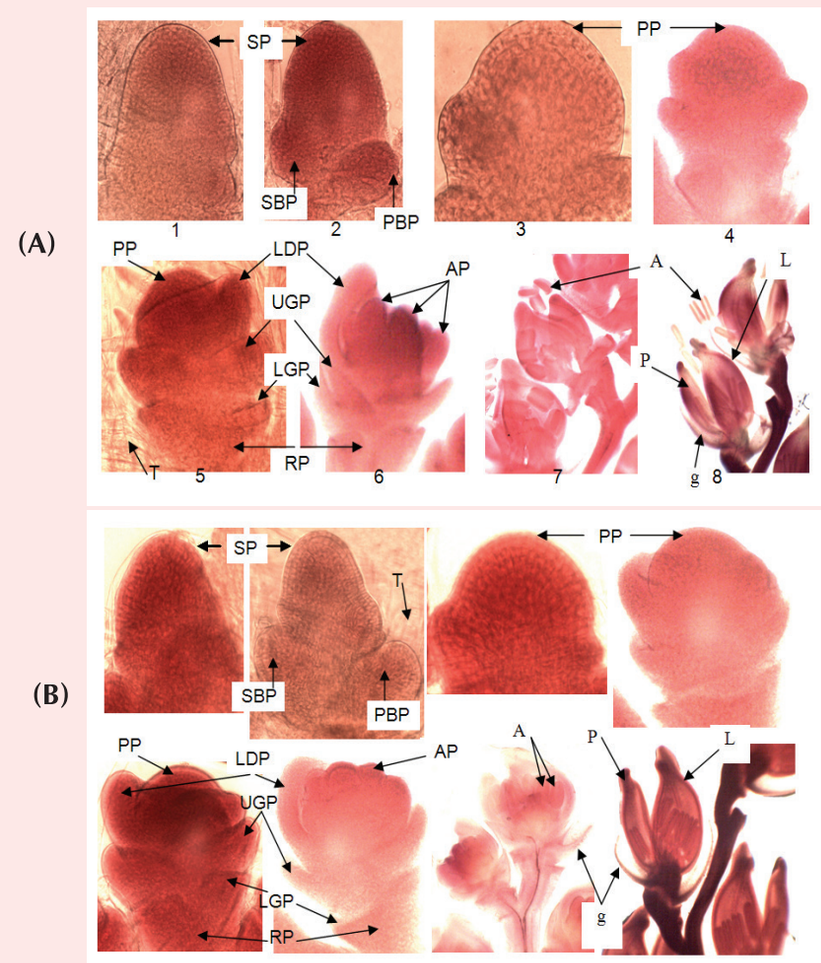

Fig. 1. The development of rice spikelets. (A) IR28; **(B)** NTCDm.

A, Anther; AP, Anther primordium; g, sterile lemmas; L, Lemma; LDP, Lodicule primordium; LGP, Lower glume primordium; P, Palea; PBP, Primary branch primordium; PP, Pistil primordium; RP, Rachilla primordium; SBP, Secondary branch primordium; SP, Spikelet primordium; T, Trichomes; UGP, Upper glume primordium.

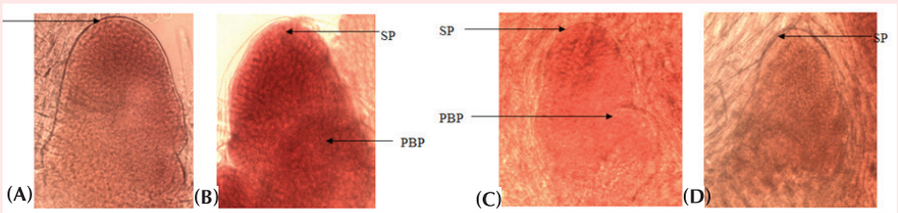

Fig. 2. Necknode differentiation stage (X100). (A) IR28; **(B)** NTCDm; **(C)** TBN; **(D)** Jasmine.
PBP, Primary branch primordium; SP, Spikelet primordium.

was consistent with the results of other studies [5].

Panicle development of aromatic and non-aromatic rice

The formation and development of aromatic rice spikelets were observed through seven stages as Matsushima's research (1970) presented.

Necknode differentiation stage:

In the necknode differentiation stage, the spikelet primordium was formed. As for the non-aromatic rice (IR28), this stage appeared from 42-44 days after sowing (DAS), but for aromatic rices, it was 50-53 DAS for NTCDm; 45-48 DAS for TBN, and 40-43 DAS for Jasmine 85, respectively. Panicle development and growth started with the neck-node differentiation and end when the pollen was fully matured.

In this phase, the part at the top bud was called the spikelet primordium, below this part was the position of the primary branch primordium (Fig. 2). The young panicle was a very small size and had protruding blocks that were not visible to the naked eye, and surrounded by trichomes. Therefore, the samples in necknode differentiation stage were very difficult to subject and difficult to be clearly visible under an optical microscope.

Branch differentiation stage:

In the branch differentiation stage, spikelet primordium continued to grow and sprout, while primary branch and secondary branch sprouts began forming. As for the non-aromatic rice (IR28), this stage occurred from 44-49 DAS, and for the aromatic rice varieties: NTCDm, TBN, and Jasmine 85, this stage occurred in about 53-59 DAS, 48-54 DAS, and 43-50 DAS, respectively.

During this period, spikelet primordium continued to grow and form primary branch primordium and secondary branch primordium. It could be seen that in the branch differentiation stage of aromatic rice (NTCDm and TBN), there was lobulation at the branch primordium (Fig. 3) while the control was not lobulated.

Spikelet differentiation stage:

At the stage of spikelet differentiation, the branch primordium continued to grow. Then, spikelet primordium, lodicule primordium, upper glume primordium, and rachilla primordium also continued to grow. This stage occurred from about 49-57, 59-67, 54-62, and 50-58 DAS, respectively corresponding to the non-aromatic rice (IR28), NTCDm, TBN, and Jasmine 85. In this phase, spikelet primordium grew to pistil primordium (Fig. 4), which then continued to grow to the pistil and stamens.

The young panicle could be seen with the naked eye for the first time in the early stages of differentiation of the secondary rachis-branches. The panicle at that time was about 0.5-0.9 mm long. A panicle that had grown 1.0 mm, had already entered the spikelet differentiation stage, and this was consistent with the results of other research [5].

After spikelet primordium in the first of the top buds developed fully such as lodicule primordium, upper glume

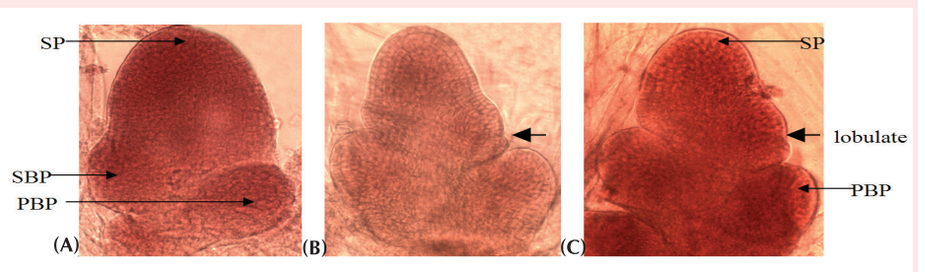

Fig.3. Branch differentiation stage (X100); samples were collected at 8:30 am. (A) IR28; **(B)** NTCDm; **(C)** TBN.
SP, Spikelet primordium; PBP, Primary branch primordium; SBP: Secondary branch primordium.

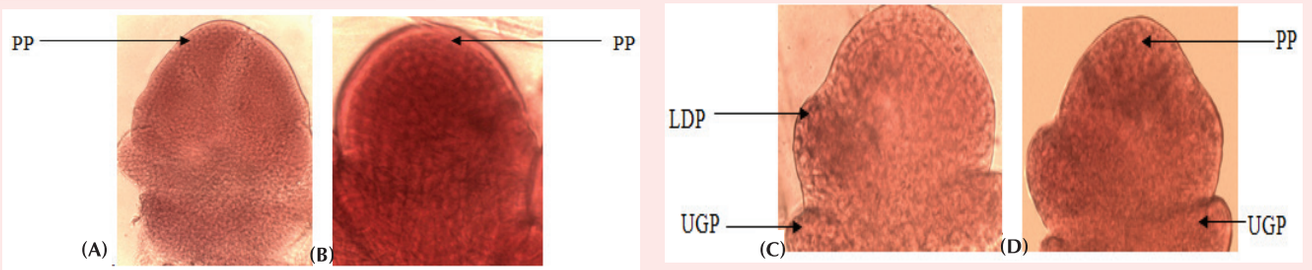

Fig. 4. Early stage (X100); samples were collected at 8:30 am. (A) IR28; **(B)** NTCDm; **(C)** IR28; **(D)** Jasmine 85.
LDP, Lodicule primordium; PP, Pistil primordium; UGP, Upper glume primordium.

primordium, and lower glume primordium, the young spikelets neighborhood continued to grow (Fig. 5). Upper glume primordium continued to grow creating sterile lemmas. Lower glume primordium continued to grow creating rudimentary glume. At this stage, rachilla primordium was formed.

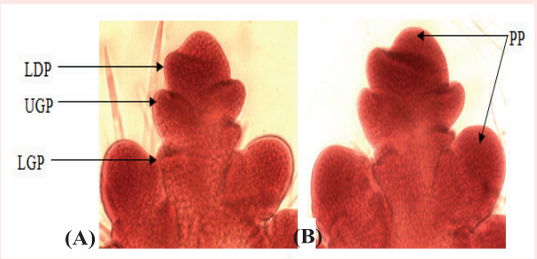

Fig. 5. Middle stage (X40); samples were collected at 8:30 am. (A) IR28; **(B)** NTCDm.
LDP, Lodicule primordium; LGP, Lower glume primordium; PP, Pistil primordium; UGP, Upper glume primordium.

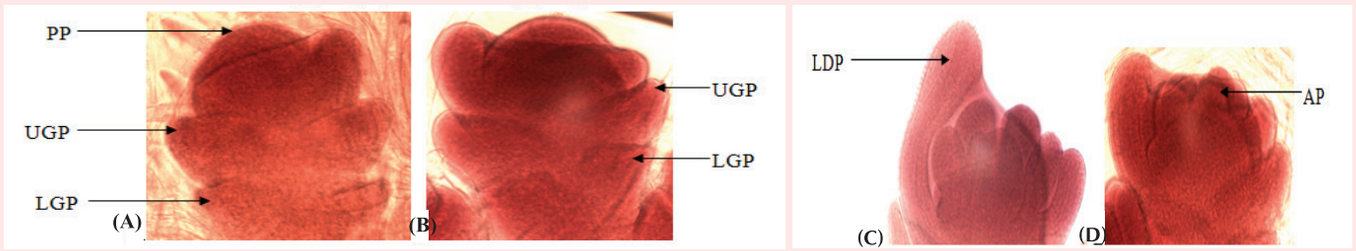

Fig. 6. Late stage (X100); samples were collected at 8:30 am. (A) IR28; **(B)** NTCDm; **(C)** IR28; **(D)** Jasmine 85.
AP, Anther primordium; LDP, Lodicule primordium; LGP, Lower glume primordium; PP, Pistil primordium; UGP, Upper glume primordium.

After LDP, UGP, and LGP, rachilla primordium continued developing and eventually created rudimentary glume, rachis, and rachilla. PP continued developing to create stamens primordium and pistil spikelet primordium. Stamens primordium continued developing to create anther primordium and filament. Pistil spikelet primordium continued to grow creating ovary, style, stigma, and pistil primordium lodicule. At this stage, lodicule primordium and upper glume primordium kept to grow. It could be seen that lodicule primordium forming a thin membrane surrounded inside anther primordium (Fig. 6).

Pollen mother cell differentiation stage:

In the stage of the differentiation pollen mother cells, the parts of the flower had been segmented and developed quite fully. The anthers insided containing pollen mother cells that were preparing to enter the meiosis stage. As for the non-aromatic rice variety IR28, this stage appeared from 57-60 days after sowing; however, it was 67-69 DAS, 62-65 DAS, and 58-61 DAS for NTCDm, TBN, and Jasmine 85, respectively.

In this stage, palea and lemma surrounded stamens and pistil (Fig. 7). The only stamens at this stage were still very

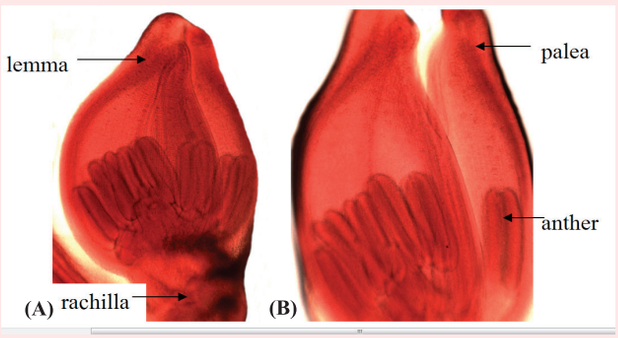

Fig. 7. A spikelet of rice (NTCDm) in Pollen mother cell differentiation stage (A) and Reduction division stage of pollen mother cell (B).

short, six anthers insided containing pollen mother cells preparing to enter the meiosis stage.

Reduction division stage of pollen mother cell:

At the stage of meiosis, pollen mother cells inside the anther started dividing and reduced to enter the process of pollen formation (Fig. 8). For non-aromatic rice (IR28), this stage appeared from 60-62 days after sowing vs aromatic rice NTCDm 69-71 DAS, TBN 65-67 DAS and Jasmine 85 63-65 DAS. The only longer stamens developed at this stage and the pollen mother cells inside anther started dividing reduced.

A bivalent number appeared in the diplotene stage showing differences (Fig. 9). As for non-aromatic rice (IR28), it had 11-12 bivalents staining, while the aromatic rice (NTCDm, TBN and Jasmine) had 7-8 bivalents staining.

The amount of homologous chromosomes was different in the aromatic rice varieties and the control variety. This might have been due to the configuration of the Rabl chromosome in the arrangement of the aromatic rice centromere location and unusual tips. Because of the findings of a report [6, 7], Rabl configuration in rice were found in the wood tissue cells and undifferentiated cells in anthers. The change of Histon and DNA methylation patterns influenced chromosome arrangements. Santos and his colleagues suggested that the DNA dimethylation in rice was caused by the non-aggregation induced chromatin configuration Rabl in the presence of abnormal tissue. These things started happening at the stage of cell division. The finding of their report [8] showed that the arrangement of chromosome

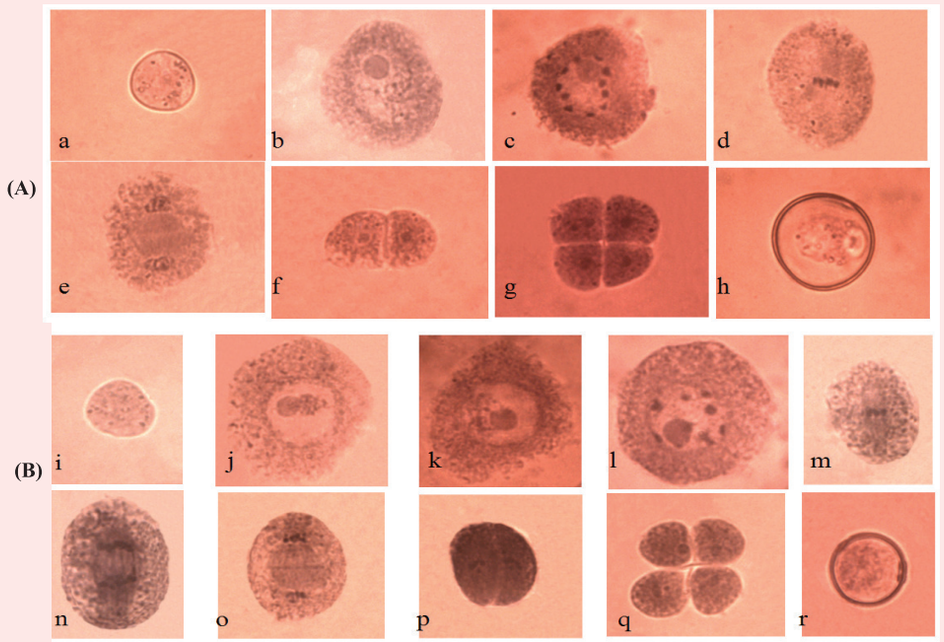

Fig. 8. Pollen formation process of IR28 (A) and TBN (B); pollen mother cell (a, i); leptotene (j); zygotene (b); pachytene (k); diplotene (c, l); metaphase I (d, m); anaphase I (n); early telophase I (e, o); telophase I (f); metaphase II (p); telophase II (g, q); pollen grains (h, r).

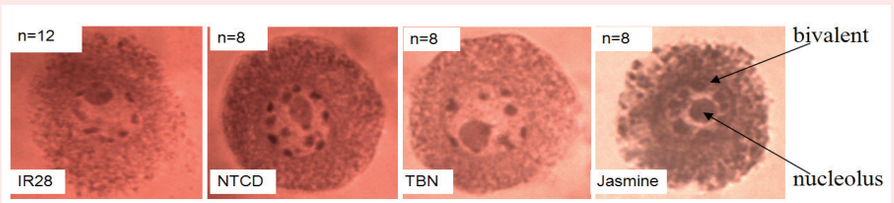

Fig. 9. Bivalent number appears in diplotene stage (X100).

territories within the nucleus exhibits dynamic changes in response to various internal and external conditions. Histone modification and DNA methylation patterns were expected to affect chromosome organization, although data on this subject is still scarce. Nevertheless, it had been shown that in rice DNA demethylation causes chromatin decondensation and induced Rabl configuration in those tissues in which Rabl was not normally presented. The structure no longer showed Histon heterochromatin. Heterochromatin's active and inactive chromatin caused no chromatin condensation. The results were of the chromosome being dye stained or faintly dye stained. The bivalent diplotene stage in aromatic rice varieties were not dye stained (or faded dye stained), they did not appear (or sometimes faintly appeared) at this stage.

Extine formation stage:

In the extine formation stage, the majority of maternal cells were split to form four spores. As for non-aromatic rice (IR28), this stage appeared from 62-64 days after sowing vs aromatic rice NTCDm 71-73 DAS, TBN 67-69 DAS and Jasmine 85 63-65 DAS. During this period, filament and style developed longer. Anther switched from white to pale yellow. Palea and lemma were thicker and stiffer.

Ripe pollen stage:

In the ripe pollen stage, pollen grains were preparing to go into the process of forming spores. As for non-aromatic rice (IR28), this stage appeared from 64-71 days after sowing while an aromatic rice NTCDm 73-80 DAS, TBN 69-76 DAS and Jasmine 85 65-72 DAS. In this phase, the anthers were yellow, inside anther contained pollen ripening process of preparing to enter form gametes.

Conclusion and suggestion

In the formation and development of rice spikelet, there were two clear points of differentiation between the aromatic rice and the non-aromatic rice: the small lobes were positioned differently and the number of homologous chromosomes stained were different. In the non-aromatic rice (IR28), there were no small lobes at the branch primordium, and the number of bivalents staining appeared with 7-8 pairs, while the opposite was viewed in the aromatic rice, there were small lobes at the branch primordium, and the number of bivalents staining appeared 11-12 pairs in diplotene stage.

This report is only the beginning. More research should continue on to understand rice flavor. It might help rice breeders for use as a monitoring tool for exact selections of aromatic rice.

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