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Sexual Plant Reproduction

Pistil hyperplasia in rice spikelets as affected by heat stress

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Summary. Sexual organogenesis in spikelets of rice (*Oryza sativa* L. cv 'Kinmaze') affected by heat stress was investigated using SEM and stereo-microscopy. Of the 243 spikelets dissected, 55.6% developed pistil hyperplasia, i.e., proliferated female organs or tissues, including multiple stigmata and/or ovaries, outgrowth of swollen parenchymatous tissues from inside the ovule, and differentiation of trichomes from ovary epidermis. Conversely, 7% of the spikelets exhibited stamen hypoplasia, represented by a decrease in the number of stamens, and only 3.7% of the spikelets showed hyperplasia, represented by an increase in the number of stamens. The morphological and structural development of the anther was disturbed, and microsporogenesis was inhibited. The shape of the anther was altered, though not perfectly, into the form of an ovary; the lemma and palea changed in form and ultimately resembled each other in shape and size. All of these changes in structure are more or less similar to those that usually result in rice spikelets subjected to cold and other environmental stresses. It was therefore concluded that rice plants show similar responses in sex differentiation in the spikelet under various environmental stresses.

Key words: Organogenesis – Pistil hyperplasia – Rice spikelet – Sex differentiation

Introduction

High temperature causes high percentages of spikelet sterility in dry season crops in many tropical and subtropical countries. For example, if the temperature exceeds 35° C at anthesis and this state lasts for more than 1 h, a high percentage of spikelet sterility occurs in a rice crop (Yoshida 1981). When cultured cells or whole organisms are exposed to elevated temperatures, they respond by synthesizing a small number of highly conserved proteins – the heat-shock proteins. These proteins are also induced by a wide variety of other stresses, and they seem to have both very general protective functions and a role in normal growth and development (Lindquist 1986). High temperature has also been seen to bring about changes in the patterns and types of hairs, epidermal cells, stomata and cuticular thickenings on stamens similar to that of a gynoecium in a temperature-sensitive mutant tomato (Sawhney and Polowick 1986).

Malformity in spikelets induced by environmental stresses or genetical factors is one of the causes for sterility in rice (Sakai 1943; Kasahara 1945; Iwaki et al. 1955; Kitamura 1955; Nunes 1964; Shimizu and Kuno 1966; Takeoka and Shimizu 1973, 1974). In such malformed spikelets the morphogenesis of the sexual organs is altered markedly with the differentiation of multiple pistils and a reduced number of stamens. Thus, floral abnormality as a cause of spikelet sterility has a great significance in rice production. This study was undertaken to clarify microscopically morphogenetic changes in rice spikelets, especially those in the sexual organs affected by heat stress.

Materials and method

Materials used in the experiments were the plants of a paddy rice (*Oryza sativa* L. cv 'Kinmaze'). Germinated seeds were sown on May 11, 1987, transplanted on June 8 in 1/5000 are Wagner pots filled with submerged soil, and grown in a glasshouse under natural daylength.

Three types of heat treatment were employed. Treatment I: the plants were treated from June 18 through to July 23, at the vegetative growth stage; treatment II: the plants were treated from July 23 through to heading time, at the reproductive stage; treatment III: the plants were treated from June 18 through to heading time, at both the vegetative and reproductive stage. To administer the heat stress we built a small room in a glass house that consisted of a steel frame covered with transparent vinyl. The temperature within this vinyl room was always above 30° C, and only the day time temperature was maintained at above 45° C. Spikelets were dissected at heading time under a stereo microscope to investigate morphogenetic changes in the sexual organs and glumes. Those

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Table 1. Morphogenetic alterations of rice sexual organs as affected by heat stress. Cultivar: 'Kinmaze', sampling date: September 14, 1987

Panicle no.	Number of spikelets dissected	Stamen		Pistil hyperplasia		
		Hypoplasia	Hyperplasia	Stigma	Ovary	Total
1	62	3	2	11	35	46
2	52	3	1	17	5	22
3	66	4	2	19	17	36
4	63	7	4	27	4	31
Total	243 (100)	17 (7.0)	9 (3.7)	74 (30.5)	61 (25.1)	135 (55.6)

spikelets showing major changes were fixed with modified Karnovsky's solution (Takeoka et al. 1983) containing 5% acrolein for 12 h at room temperature, and dehydrated in acetone. The dehydrated spikelets were then dried with a critical point drier (Hitachi CP-2). The pieces from malformed organs of the dried spikelets were then mounted on polished brass stubs with bilateral adhesive tape and examined with SEM(JEOL JSM-F) operated at 15 KeV.

Results

Table 1 shows the frequency of different types of morphological abnormalities resulting from heat treatment III. Of the 243 spikelets examined, 55.6% developed different types of pistil hyperplasia, i.e., proliferated female organs or tissues including multiplicated stigmata (Figs. 4, 5, 10) and/or more than one ovary (Figs. 6–8, 11–12), outgrowth of swollen parenchymatous tissues from inside the ovule (Figs. 9, 17), and differentiation of the trichomes from the ovary epidermis (Fig. 18); 7.0% developed stamen hypoplasia, i.e., a decrease in the number of stamens in the spikelet; 3.7% developed stamen hyperplasia, i.e., an increase in the number of stamens in the spikelet. The gross morphological and structural development of the anther was disturbed, resulting in the failure of microsporogenesis (Fig. 16), and the shape of anther was altered, though not perfectly, into the shape of an ovary (Fig. 15, arrows). The morphology and development of the lemma and palea also changed considerably and these turned into structures having a similar shape and size (Fig. 2).

Spikelets grown under heat treatment II also showed similar pistil hyperplasia and stamen hypoplasia; however, spikelets grown under treatment I showed almost no abnormalities in sex differentiation.

Discussion

The most observable morphogenetic change in the spikelets affected by heat stress that were investigated in this study was that more than 50% of the spikelets developed pistil hyperplasia. Pistil hyperplasia in rice spikelets has also been observed under other environmental stressful conditions; for instance, paddy and upland soil conditions (Kitamura 1955), low water temperature (Shimizu and Kuno 1966), stressful organic matter in the soil (Takeoka et al. 1989), cultivation of paddy rice under upland conditions (Takeoka et al. 1989), supply of exogenous gibberellin (Shimizu and Takeoka 1965) and treatment by a chemical hybridizing agent (Takeoka et al. 1990). Genetical factors can also be responsible for pistil hyperplasia (Takeoka and Shimizu 1973, 1974; Takeoka et al. 1987). The main cause of sterility in rice spikelets as a result of cold injury is male abortion, e.g., microsporogenesis failure or anther dehiscence (Satake and Hayase 1970). In this study, with respect to stamen abnormality, stamen hypoplasia occurred at a higher frequency than stamen hyperplasia. Stamen hypoplasia causing abnormal organogenesis or histogenesis also occurs in spikelets as a result of straighthead affected by stressful organic matter in the soil (Takeoka et al. 1989), drought (Takeoka et al. 1989), mutation (Takeoka and Shimizu 1973), and chemical treatment (Takeoka et al. 1989). In a temperature-sensitive mutant tomato temperature stress was also found to have formative effects on the surface features of the stamens: under low temperatures, features resembling normal stamens were produced, while under high temperature all features resembled those of a gynoecium (Sawhney and Polowick 1986).

Based on their observations on the development and morphogenesis of genetically proliferative spikelets, Takeoka and Shimizu (1979) suggested that the occurrence of pistil hyperplasia and stamen hypoplasia in rice spikelets subjected to different environmental stresses or having genetic disorders might be said to be universal. They suggested there are three forms of proliferative spikelets: S-type proliferation, a spikelet having one or more spikelets in it; P-type proliferation, a spikelet having a proli-

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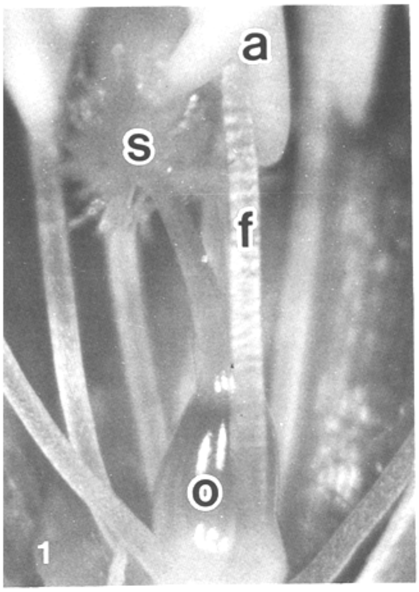
Figs. 1–9. Side views, as observed by stereo-microscopy, of the inside of spikelets, with focusing centered on the pistil and stamens: A normal plant (Fig. 1) and *plants affected by heat stress*. Figs. 2–9. **Fig. 1.** Normal pistil having two stigmata (*s*). A normal rice spikelet has six stamens, each of which is composed of an anther (*a*) and a filament (*f*). *o* Ovary, ×38. **Fig. 2.** A spikelet showing that the lemma (*l*) and palea (*p*) have changed to have a similar shape and size. The near side of both lemma and palea has been removed. ×13. **Fig. 3.** An enlarged view of the basal portion of Fig. 1 showing that a pair of filmy and spear-shaped glumes (*arrows*) have differentiated. These glumes also differentiate in spikelets affected by, e.g., low temperature and exogenous gibberellin. ×23. **Fig. 4.** A pistil with three differentiated stigmata (*arrow*). ×32. **Fig. 5.** A pistil with four differentiated stigmata (*arrows*) and two adhered ovaries. Note that stamens (*st*), in contrast to the pistil, grew poorly. ×32. **Fig. 6.** Two differentiated pistils in a spikelet: one is similar to the pistil in Fig. 5 (at right); the other (at left) is similar in shape and size to a normal rice pistil. ×32. **Fig. 7.** Two differentiated pistils in a spikelet, both of which are similar in shape and size to a normal spikelet. Note that the stamens shown in the figure grew poorly. ×32. **Fig. 8.** Two differentiated pistils in a spikelet, with stamens growing extremely poorly. ×30. **Fig. 9.** A pistil having three stigmata and round, swollen tissue (*arrow*) at the junction of the style and ovary. ×32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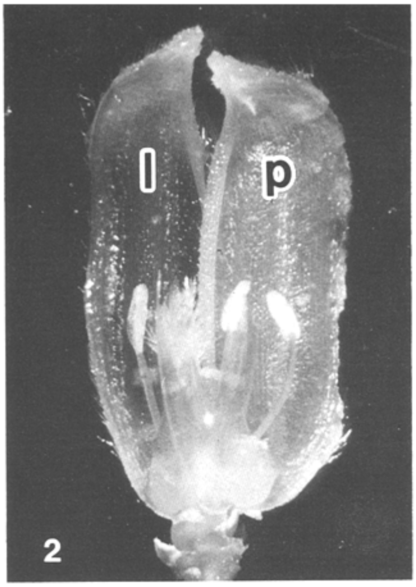
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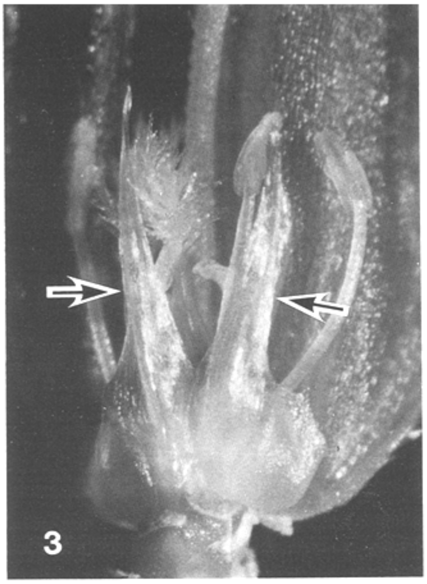
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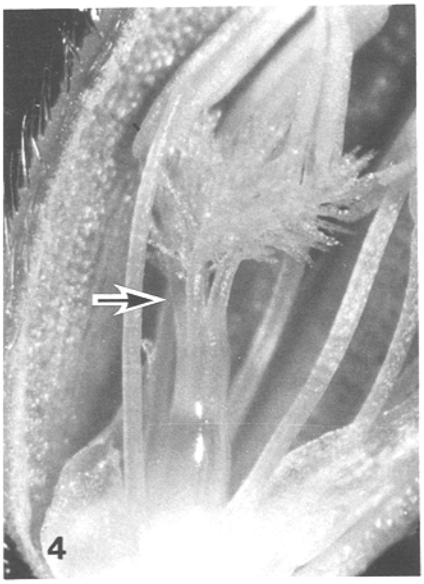
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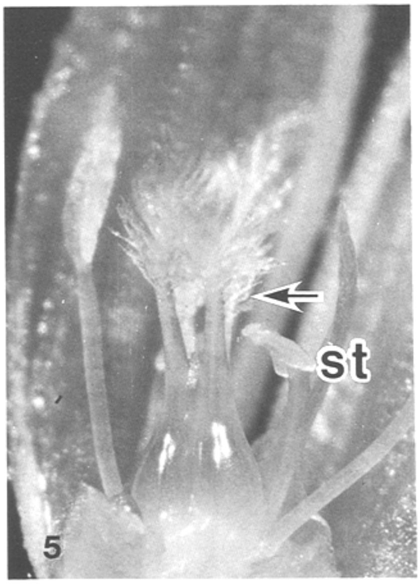
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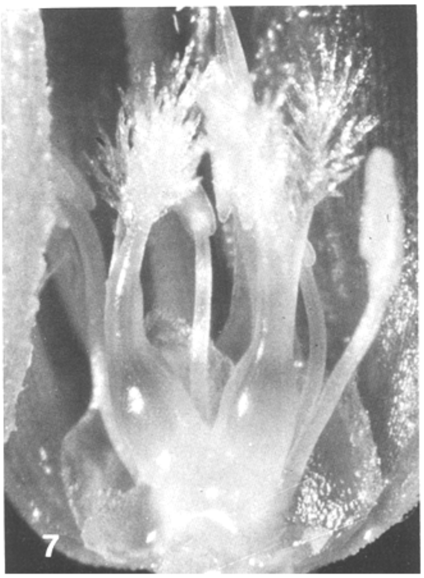
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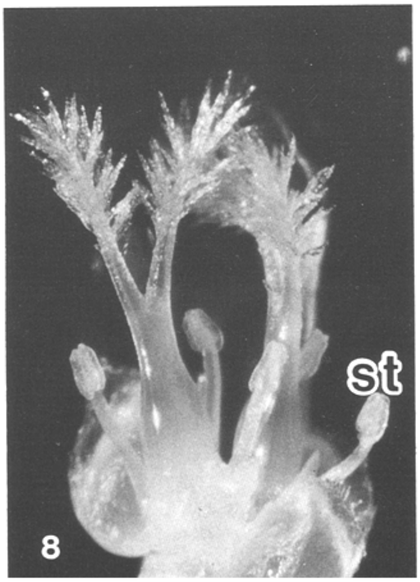
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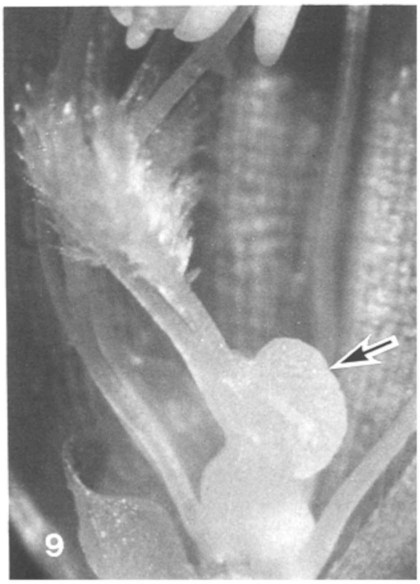
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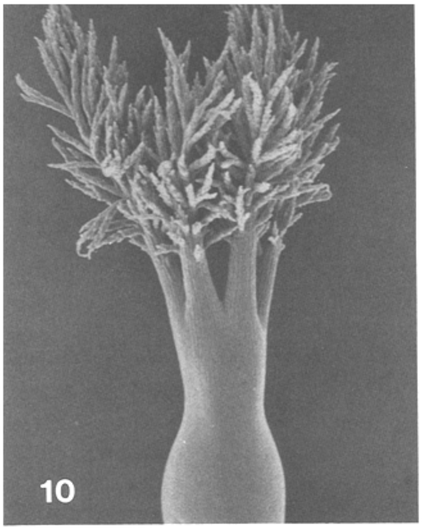
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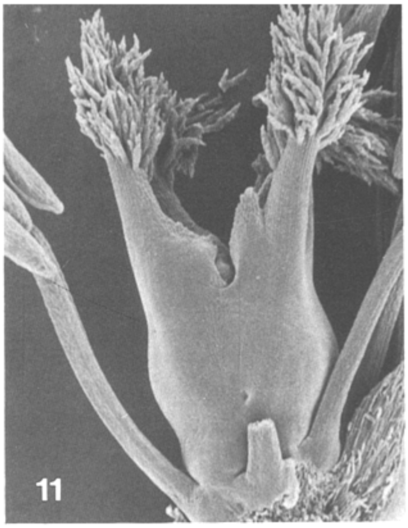
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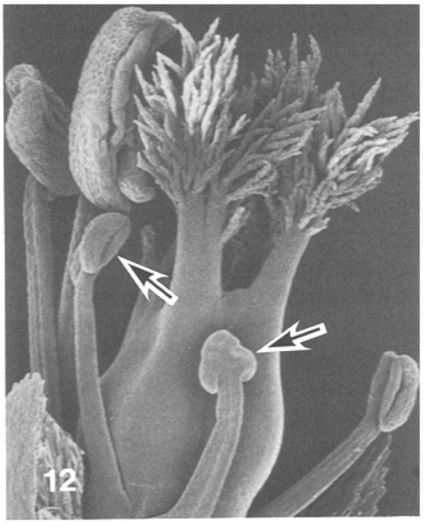
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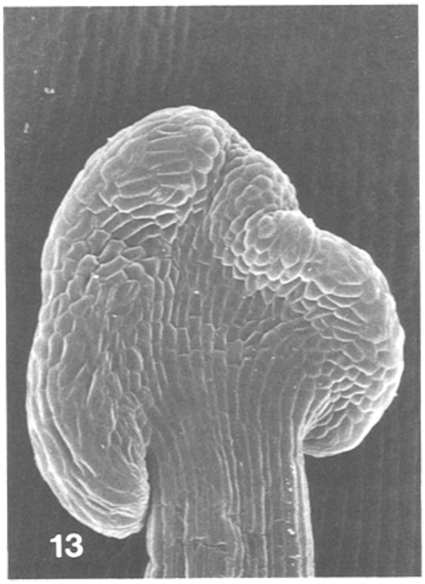
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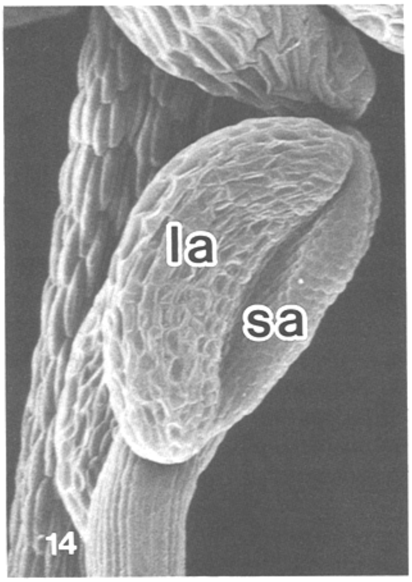
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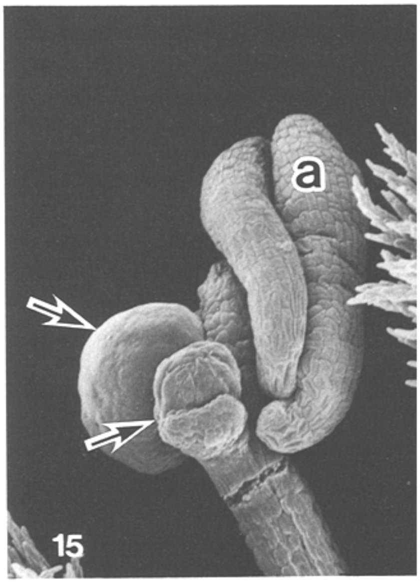
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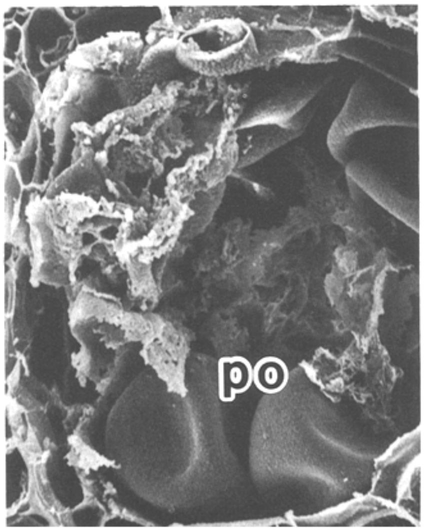
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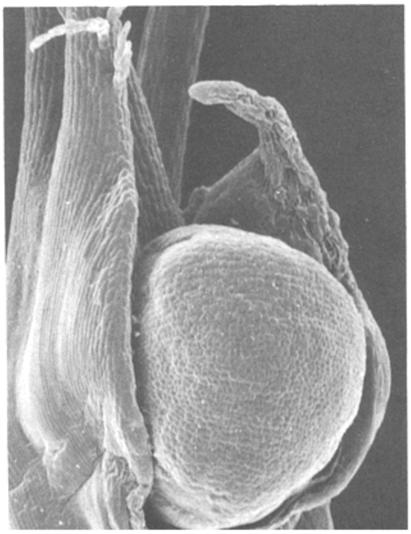

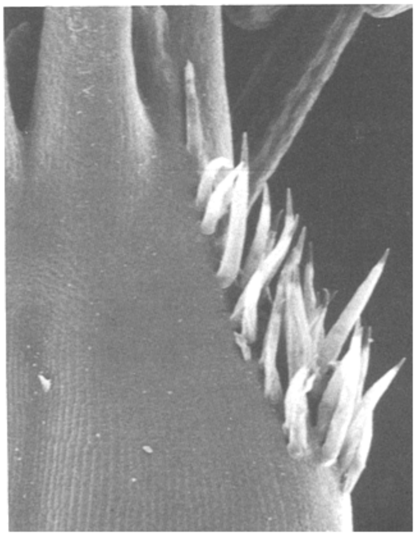

ferated pistil and/or stigma; L-tpe proliferation, a spikelet having one or more leafy shoot in it. These three types of proliferation are not independent, but represent three successive stages of proliferation, the final stage being the leafy shoot type. These proliferations are always accompanied by one or more of the following four stages of incompleteness in floral organs. Stage I: lack of floral organs – the floral apex grows upward and produces a spikelet on its head. Stage II: complete or partial degeneration of stamen with normal pistil. Stage III: increment in pistil-like organs. Stage IV: degeneration of stamen and pistil and development of the spikelet into a leafy shoot. Takeoka and Shimizu (1979) consider stage I to be the S-type, stages II and III to be the P-type and stage IV to be the L-type. Under the above-stated considerations the stamen hypoplasia and pistil hyperplasia observed in this study represent stage II and stage III, respectively.

The biochemical basis of these heat stress malformities are still not clearly understood, but the possibility that heat shock protein is synthesized in the spikelet of rice plant during the stress period and that this protein contributes to the regulation of pistil hyperplasia and stamen hypoplasia cannot be ruled out.

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Figs. 10–18. Side views (Figs. 10–15, 17, 18) and tranversely cut surface view (Fig. 16), as observed by SEM, of rice sexual organs affected by heat stress. **Fig. 10.** A pistil with four differentiated stigmata, the ovary of which clearly does not adhere as in the pistil shown in Fig. 5. × 38. **Fig. 11.** At first glance, one pistil having four stigmata; however, this resembles the pistil in Fig. 5, at the base of which two ovaries adhere to form what appears to be one ovary. × 38. **Fig. 12.** A multistigmatal pistil and five stamens (*arrows*) growing poorly, the degree of which varies on account of the stamen. × 38. **Figs. 13, 14.** Enlarged views of the anthers shown in Fig. 12. **Fig. 13** shows a posterior view of the anther, the size of which is very small in comparison with the diameter of the filament. × 219; **Fig. 14** also shows a very small anther although it differentiated to a large anther locule (*la*) and small anther locule (*sa*). × 219. **Fig. 15.** Tip of a stamen showing a round ovary-like tissue (*arrows*) that differentiates beside the small anther (*a*). × 93. **Fig. 16.** Internal view of an anther showing that the shape of the exine of each pollen grain (*po*) became dented, which indicates the accumulation of contents within to be minimal × 767. **Fig. 17.** Outgrowth of swollen parenchymatous tissues from inside the ovule. × 87. **Fig. 18.** Slender trichomes have differentiated from the epidermis on the shoulder of the ovary. × 86

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