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Morphogenetic Alterations of Rice Floral Organs as affected by Spray of Chemical Hybridizing Agent HGR-626*

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Abstract : Morphogenetic alterations of rice spikelets grown under the influence of applied chemical hybridizing agent HGR-626 was studied using SEM and stereo microscope. Either a 10 ppm or 50 ppm solution of HGR-626 was sprayed at the spikelet primordium differentiation stage (Stage I), and in the middle of the meiosis stage (Stage II), respectively. Spikelets treated at Stage I showed such changes as : (1) spikelet and its component organs became smaller than control, (2) anthers of which bended toward axis, (3) no pollen mother cell differentiated in them, or (4) microspores with anther tissues ceased to develop, and, (5) exine of microspores developed irregularly to interconnect each other. Almost no content accumulated in them. On the contrary, 56.4% of pistils investigated in this study proliferated their ovaries and/or stigmata. Change of sex initiation namely pistil hyperplasia and stamen hypoplasia in rice spikelet as shown in this study indicated a similar tendency with the pistil hyperplasia and stamen hypoplasia induced by stressful environments studied previously. Skikelets treated at stage II showed no morphogenetic change as spikelets treated at stage I. This difference was considered to have occurred from the difference in developmental stage of apical meristem in spikelet primordia when the chemical was applied.
Key words : Chemical hybridizing agent, HGR-626, Morphogenesis, Pistil hyperplasia, Rice, SEM, Sex initiation, spikelet.

化学交雑剤 HGR-626 の散布によるイネ花器官の形態形成的変化：武岡洋治**・伊藤雅章**・山村三郎***・坂　斉**** (**名古屋大学農学部, ***北興化学, ****農業生物資源研)

要　旨：化学交雑剤 HGR 626 の散布の影響によるイネ花器官における形態形成の変化を走査電子顕微鏡 (SEM) および実体顕微鏡で観察し，各種環境ストレスで誘発される形態形成の変化と比較した．水稲日本晴を 1/5000 a ポットで土耕し，穎花始源体分化期（処理 I 期），または減数分裂盛期（処理II期）に，10 または 50 ppm の同剤水溶液を茎葉散布した．出穂期に FAA で固定した小穂を実体顕微鏡で解剖するとともに，アセトン脱水・臨界点乾燥・金コーティングの後 SEM で観察した．処理 I 期の小穂では両濃度区ともに (1) 小穂器官が標準より矮小化し，内側に湾曲した葯の割断面では，(2) 花粉母細胞が正常に分化せず柔細胞のみが放射状に配列しているか，(3) 小胞子と葯壁の形成が途中で停止しているもの，(4) 花粉外殻の形状が異常で内容物も充実不十分なもの，または (5) 花粉が隣同士癒着しているものなどが認められた．雌ずいでは組織・器官の形成阻害は見られず，解剖小穂 433 個の 56.4%が柱頭または子房を増殖していた．処理II期の小穂ではこの種の変化はなく，同剤供与下での小穂形態形成は処理時期により様相を異にした．性発現における雌性化傾向は環境ストレスにより発現する既往の結果と類似しており，同剤の影響下での小穂形態形成もこれらによる場合と同様の経過を辿ることが明かになった。処理II期の小穂に処理 I 期のような変化が生じなかったのは，処理時期における小穂始源体の頂端分裂組織における形態形成段階の相違によると考えた．

キーワード：イネ，HGR-626，化学交雑剤，形態形成，雌ずい増生，小穂，性発現，走査電子顕微鏡．

It is well known that mophogenetic changes take place in reproductive organs in rice spikelets due to environmental elements[1,2,10,11,16,17,18,20,21] or genetic causes[18,19]. Among those disorders, many cases indicate that spikelets have a tendency to multiplicate their female organs as compared with stamens to retrograde, which implies hyperplasia and hypoplasia[24] in pistil and stamens respectively.

HGR-626 (=RH-531) have been reported to act as a gametocide or chemical hybridizing agent in rece[7,14,22,25]. This study was undertaken to clarify, using SEM and stereo microscope, the morphogenesis of rice spikelet, spe-

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Table 1. Morphogenetic alterations of rice floral organs as affected by spray of chemical hybridizing agent HGR-626 in 10 ppm.

Panicle No.	Number of spikelets dissected	Lodicule		Stamen		Pistil Hyperplasia		
		Hypo-plasia	Hyper-plasia	Hypop-lasia	Hyper-plasia	Stigma	Ovary	Total
1	97	0	5	55	0	65	0	65
2	127	0	11	74	0	83	3	86
3	76	0	4	43	0	23	2	25
4	79	0	2	59	0	40	6	46
5	54	0	3	29	0	19	3	22
Total	433	0	25	210	0	230	14	244
	(100)	(0)	(5.8)	(48.5)	(0)	(53.1)	(3.2)	(56.4)

Cultivar : Nipponbare.
HGR-626 : Sodim-1-(p-chlorophenyl)-1,2 dihydro-4,6-dimethyl-2-oxo-nicotinate.

cially both of stamen and pistil as affected by sprayed HGR-626, and compare the morphogenetic changes with them as affected by various environmental stresses.

Materials and Method

Materials used in this study were a paddy rice (*Oryza sativa* L.) cv. Nipponbare. Germinated seeds were planted on May 22th. When plants grew at seventh leaf stage on June 11th, they were transplanted to 1/5000 a Wagner pots filled with submerged soil mixed 3 g of compound fertilizer (N : P : K = 14 : 16 : 14) in every three plants per pot. Either a 10 ppm or 50 ppm of aqueous solution of HGR-626 (Sodium-1-(p-chlorophenyl)-1, 2-dihydro-4, 6-dimethyl-2-oxo-nicotinate) was sprayed to leaves and stems at the stage of (I) spikelet primordium differentiation on July 27th, and (II) meiosis in the stamen on August 11th. Panicles and spikelet were fixed at the stage of heading with FAA (mixed solution of formalin, acetic acid, and ethyl alcohol) for more than 24 hours at room temperature. Fixed materials were rinsed with 0.05 M of phosphate buffer adjusted to pH 7.2, dehydrated in acetone, dried with a critical point drier (HITACHI CP-2), cut by a razor, and mounted on polished brass stubs with bilateral adhesive tape. The materials were then coated with gold for 5 min at 7 mA by an ion spatter (EIKO IB-51), and examined with SEM (JEOL JSM-F7) operated at 15 KeV.

Results

The spikelets treated with HGR-626 at the spikelet primordium differentiation stage were observed to show varieties of morphological and histological malformities as follows; The spikelets were smaller than control in size as shown in Fig. 1, namely, the length of the treated spikelet was 4.5 mm versus 7.8 mm in control. The ratio of spikelet width and length was 1 : 1.9 and 1 : 2.4 in the treated and control, respectively.

Both stamens and pistil also became smaller in the treated spikelet than control, and the anther bended to inside due to abortion of small anther-loculi as shows in Fig. 2, which shows that the whole size of the anther was reduced specially in length. Furthermore, both small anther-loculi ceased to develop at the very early stage. The ratio of width to length of the anther was 1 : 1.4 in contrast with 1 : 2.8 in normal anther. According to transversely cut surface view of anther in these stamens, only parenchymatous cells differentiated as shown in Fig. 3, which shows that none of the pollen mother cell (PMC) differentiated except for radiated parenchymatous cells. An identical result was obtained for endothecium, middle layer and tapetum composing anther wall-layers. Fig. 4 shows a SEM view of transversely cut surface of an anther loculus treated with HGR-626 indicating no microspore or young pollen except cytoplasmic aggregations in it.

The shape of pollen changed irregularly as

shown in Fig. 5, which shows that the shape was not round as usual and no content accumulated in it. Exine development also greatly changed as Fig. 6. It shows a SEM view of cut surface of exine to interconnect neighboring pollens, the thickness of which is not uniform, and gills developed as indicated by arrows in the figure. As shown in Table 1, 48.5% of the total 433 spikelets dissected in the study dicreased their number of stamens below usual six, and no spikelet increased the number above six. This indicates that under the influence of HGR-626 stamens showed hypoplasia in their morphogenesis. On the other hand, pistil showed a contrastive change with stamens in its organogenesis; namely, 56.4% of 433 pistils dissected proliferated more ovaries and/or stigmata as shown in Table 1, which is a result of spikelets applied with 10 ppm. Fig. 7 shows a SEM view of the pistil treated with HGR-262 having an extra stigma. Moreover, pistil showed parthenocarpic development of the ovaries forming a pseudocarp under HGR-626 treatment as shown in Fig. 8. Therefore, the pistil showed hyperplasia in its morphogenesis under the influence of HGR-626. Such morphohistological changes were not observed in spikelets treated at stage II.

Discussion

A SEM observation made clear that stamens and pistil showed a contrastive pattern on their morphogenesis under the influence of exogenous application of HGR-626; stamens reduced their organo-, and histo-genesis, resulting in a failure of the pollen mother cell and tapetum to differentiate, or to be supressed to form exine and starch. Pistil, on the contrary to the stamens, promoted its morphogenesis, which proliferated stigma and/or ovary, and formed pseudocarp.

A variety of studies pointed out that morphogenetic disorders were induced in spikelets by environmental or genetic causes, for example, low-[17], or high-temperature[20], drought[2], straight head[11], disease infection[1], treatment with gibberellin[16], excess nitrogen application[10], and induced mutation[18,19]. Many of these cases indicate the promotion of pistil organogenesis showing hyperplasia or pistillody, as compared with stamens which retrograde indicating hypoplasia. Results of the present study showed a similar pattern of morphogenetic changes in sex organs due to those of stressful environments as mentioned above.

Effects of gametocides have been investigated when used as a chemical hybridizing agent, on floral development[4], microsporogenesis[3,5,13], male sterility[8,9,12,14,15,22,23,25], or anther dehiscence[6,13]. The investigations on the effect of chemical hybridyzing agents on plants indicated that the sterility was induced by

Abbreviations;
e: exine, **l**: lemma, **o**: ovary, **p**: palea, **pc**: parenchymatous cell, **PMC**: pollen mother cell, **po**: pollen, **s**: stamen, **t**: tapetum.

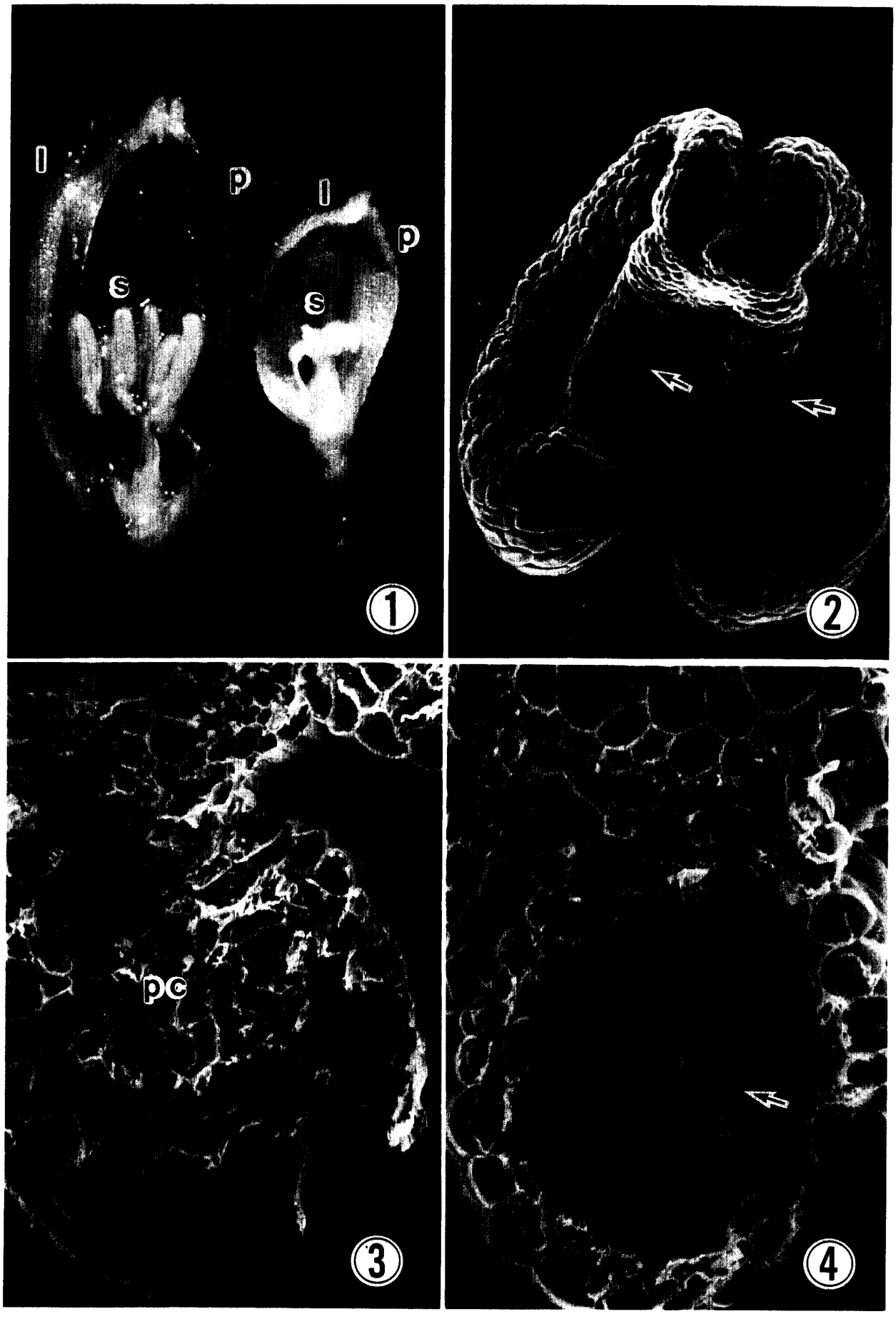
Explanation of figures (Figs. 1-4)

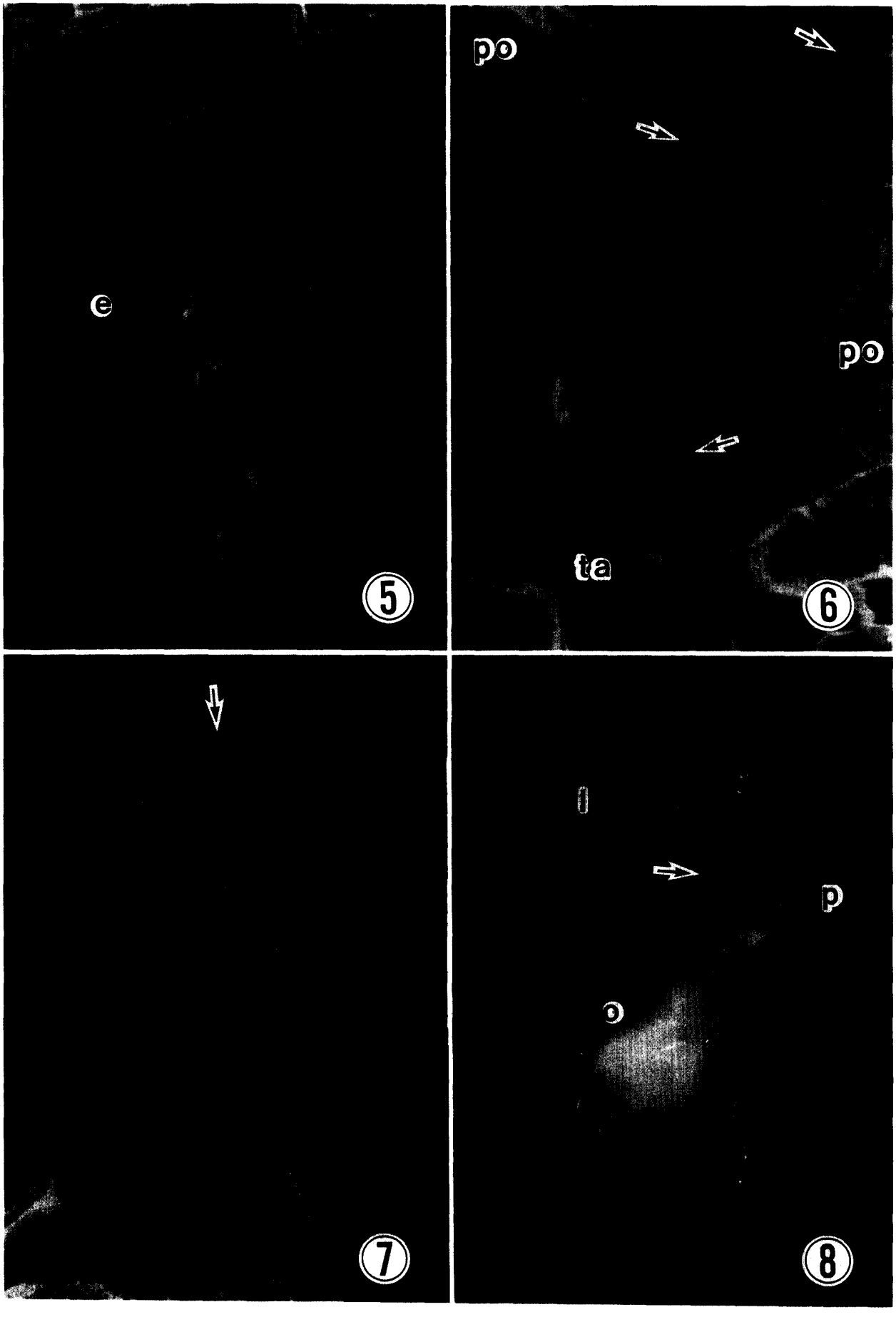
Fig. 1. Side view of untreated spikelet (left), and spikelet treated with HGR-626 (right) observed by stereo-microscope, which shows that not only the outer shape of the spikelet but also stamens (s) became smaller especially in length in treated spikelet than normal. This side of lemma (l) and palea (p) in both spikelets were removed for the convenience of investigation (×10).

Fig. 2. Side view of an anther treated with HGR-626 observed by SEM, showing that whole shape of the anther shortened especially in length, and both small anther-loculi (arrows) ceased to develop at very small stage (×290).

Fig. 3. Transversely cut surface view of an anther-lobe treated with HGR-626 observed by SEM, showing that no pollen mother cell (PMC) differentiated except radiated parenchymatous cells (pc), however, no three layers of anther wall composed of endothecium, middle layer, and tapetum were observed to differentiate (×750).

Fig. 4. Transversely cut surface view of an anther-loculus treated with HGR-626 observed by SEM, showing no microspore or pollen inside of the loculus except cytoplasmic aggregations (arrow) (×750).





mainly tapetum persistence[13,15]. It was clarified that the gametocides RH531 and RH532 were highly potent and act directly on the genetic material of wheat to bring about various gross pollen abnormalities, and thus sterility, and that not only was the tatetum shown to be persistent but a total disruption of meiosis and pollen exine differentiation was observed[15]. A chemical hybridyzing agent RH0007 was considered to appear to interfere with the polymerization of carotenoid precoursors into the exine wall and ubish bodies, rather than interfering with the synthesis of the precursors through a study of pollen development in wheat using transmission electron microscopy (TEM)[13]. However, few study have reported on the morphogenetic change of sexual organs as shown in this study. Prior to this study, we undertook a SEM study on the normal course of spikelet morphogenesis in rice focusing mainly on developmental changes in size and form of the apical meristem of spikelet primordium, correlation in the course of growth among the stamens, sterescopic arrangement of organs composing spikelet, and developmental changes in surface structure of anther[21]. As made clear in this study, the rice plants treated at stage I were just on the way to initiate spikelet component organs. However, plants treated astsage II after one week of stage I have already finished to initiate their organs. This developmental difference at the apical meristem resulted the difference in the change of spikelet morphogenesis as affected by the chemical agent. A histological observation is undertaken on momphogenetic changes of pollen and pistil due to the same agent as used in this study.

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Explanation of figures (Fig. 5—Fig. 8)

Fig. 5. Cut surface view of pollen exine (e) treated with HGR-626 observed by SEM, showing that the shape was not round as usual but irregular and no starch grain was observed to accumulate in it (×2400).

Fig. 6. Cut surface view of pollen exine treated with HGR-626 observed by SEM, showing that neighboring pollens (po) were interconnected by exine, thickness of which is irregular and has small gills (arrows). Granular contents in pollens scarcely accumulated in them (×2200).

Fig. 7. Side view of pistil treated with HGR-626 observed by SEM which differentiated one more stigma (arrow) than usual two (×65).

Fig. 8. Side view of spikelet treated with HGR-626 observed by stereo-microscope, which shows that the ovary (o) developed considerablly especially its basal portion. Arrow indicates withered stigma. This side of the lemma (l) and pales (p) was removed for the convenience of investigation (×20).

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