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Morphogenetic Alterations of Spikelets on a Straighthead Panicle in Rice*

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Abstract : A study was undertaken to clarify microscopically the characteristics of morphogenetic alterations in rice spikelets on a straighthead panicle. Plants of an upland rice (*Oryza sativa* L. cv. H4) were grown by the method "Onae Ishokuho" developed by Matsuo et al. to induce straighthead symptoms. Spikelets fixed with Formaline-acetic acid-alcohol at heading time were dissected under a stereo microscope to investigate morphogenetic changes of sexual organs—the pistil and stamens—and glumes. Some of the spikelets having principal changes were examined by scanning electron microscope (SEM). Of the total 407 spikelets dissected, 93.9% lacked in both sexual organs and 18.9% developed spikelet-type (S-type) proliferation, in which instead of normal sexual organs small spikelet(s) differentiated. A small protuberance viewed by stereo microscope at the site of pistil was identified by SEM to be the same as normal spikelet primordium. Of the total spikelets, 3.7% developed pistil hyperplasia to have differentiated more stigma(ta) or pistil(s) than the usual number. Almost all of the spikelets changed the shape of their lemma and palea, and in 40.0% of the total spikelets the palea developed. On the other hand, glumes were increased in 11.5% of spikelets. Spikelets lacking in sexual organs were considered to have possibility to develop any kind of proliferation. It was concluded that initiation of pistil hyperplasia and S-type proliferation were main charactreristics in morphogenetic changes of sexual organs induced by straighthead in the present study. Similarity of the changes with other morphogenetic changes by different environmental stresses and its significance were discussed from the view point of spikelet morphogenesis and vegetative proliferation.
Key werds : Morphogenesis, Pistil hyperplasia, Proliferation, Rice, Scanning electron microscope (SEM), Spikelet, straighthead.

イネにおける青立ち穂上小穂の形態形成的変化：武岡洋治**・筒井芳郎**・松尾喜義***（**名古屋大学農学部・***四国農業試験場）

要　旨：松尾らが開発した大苗移植法により誘発した青立ち症イネ小穂における形態形成の変化の特徴を明らかにするために実体顕微鏡および走査電子顕微鏡（SEM）による観察を行った．1987年四国農試で陸稲H4を本法により栽培して得た青立穂を出穂期に固定し，実体顕微鏡下で生殖器官の変異の態様を解剖調査するとともに，主要な変異の小穂をアセトン脱水・臨界点乾燥・金コーティングの後15 keV SEMで観察した．①内部に生殖器官を欠失した小穂は全解剖小穂数（407）の93.9%に上ったが，18.9%の小穂が小穂を反復して分化した小穂反復型貫生体を発現していた．実体顕微鏡下で微小な突起に見えたものは明かに小穂始源体であることをSEMにより確認した．②3.7%の小穂で雌ずいが多柱頭化または群生していた．③殆ど全ての小穂で外穎と内穎とが変形して鉤合不能になっていたが，全体の40.0%の小穂で特に内穎が退化し，11.5%の小穂で護穎，外穎ないし内穎が増加していた．生殖器官を欠失した小穂は内部に微小突起が認められたことから，生育条件により新たに貫生体を発達させる可能性があると考えた．誘発青立ち症イネの生殖器官における形態形成の変化は，雌ずいの増生と小穂反復型貫生体の発現を特徴とし，他の環境ストレスによる変化と共通性をもつことを明らかにした．小穂器官の形態形成と性発現の面から貫生発達の意義を考察した．
キーワード：青立ち症，イネ，貫生，形態形成，小穂，雌ずい増生，走査電子顕微鏡．

Rice plants grown in the paddy fields converted from the upland fields cause a variety of abnormalities in growth[8,9,10]. Occurrence of the growth abnormalities is more frequent up to the first three years of conversion of the cultural condition. Kitamura[8] observed that the aberrations mainly occurred in the panicle and sexual organs, the pistil and stamens, of the plants with an extraordinarily wide range of variations, and pointed out that the occurrence of these aberrations were closely related to the soil conditions and soil texture, especially the water logging capacity of the soil. According to him, no extreme abnor-

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malities were observed on sandy loam soil; however, on loam or clay soil more variation in the abnormalities were observed even after two to three years of conversion of cultural conditions from upland to paddy. Considering the symptoms and conditions of occurrence, these aberrations were bracketed with the "Straighthead", a physiological disease usually found in the southern part of U.S.A.[25]. In order to find out the cause and control method of the abnormal development of rice plants, studies[5,11,12,13] were made using the affected upland fields or soils, artificially changed soil, and different environmental conditions, and it was concluded that straighthead of rice plants would occur not only in a paddy fields converted from a upland one but also in common paddy fields in which exess organic matter, such as, barley straws had been applied.

Sexual organs in rice spikelets are known to change their morphogenesis to multiplicate pistil and to retrograde stamens under the influence of different environmental elements[1,2,3,4,6,7,15], or genetic causes[14,16,18]. This study was made to clarify microscopically the morphogenetic changes of spikelets, especially sexual organs on a straighthead panicle induced by excess soil organic matter. The similarity of the abberations with those induced by other environmental stresses in rice sexual organs was discussed.

Materials and Method

Materials used for experiment in this study were the plants of upland rice (*Oryza sativa* L. cv. H4). Plants were grown by a method "Onae Ishokuho" developed by Matsuo et al.[13] to induce straighthead experimentally. Seeds were sown on 2 June 1987 in 1/10000 a pots filled with ordinary paddy soil, and seedlings were transplanted on 18 July 1987 to 1/5000 a pots filled with 4 kg/pot of paddy soil sampled from a paddy field at Osato, Saitama prefecture where straighthead symptoms were apt to occur, and the pots were then added with 10 g/pot of soluble starch. Each pot was kept on constant submerged condition. Panicles were fixed with FAA (a mixture solution of formalin, ethyl alcohol and acetic acid, 5 : 90 : 5) at heading time at the end of September, and spikelets were dissected under a stereo microscope to investigate morphogenetic change of sexual organs and glumes. Spikelets having principal changes were dehydrated with acetone at room temperature, and dried with a critical point drier. The dried pieces were then mounted on polished brass stubs with bilateral adhesive tape, coated with gold by an ion coater (EIKO IB-5), and examined with SEM (scanning electyron microscope, JEOL JSM-F7) operated at 15 keV.

Results

Fig. 1 shows a straighthead panicle investigated in the study. Almost all of the spikelets, being deformed especially at the tips of lemma and palea as shown by arrow, changed to a beaked shape. The types of morphological abnormalities and their frequencies in the dissected spikelets as observed under stereo microscope are presented in Table 1. Of the total 407 spikelets, 93.9% were lacking in both the sexual organs. Figs. 9 and 10 shows the spikelets lacking in sexual organs. In each of

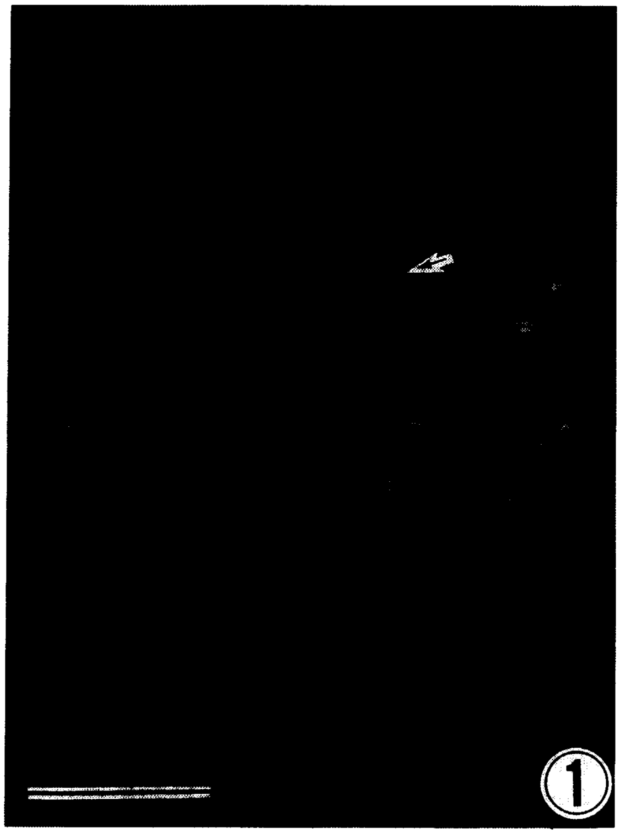

Fig. 1. A picture of the straighthead panicle. Allmost of the spikelets deformed of their lemma and palea having no jointing wings between lemma and palea. Arrow indicates beaked lemma. Bar indicates 5 cm.

Table 1 Morphogenetic alterations of rice spikelets on a straighthead panicle.

Panicle No.	Total spikelets dissected	Hypoplasia in glumes	Hyperplasia in glumes	Hyperplasia in pistil	Lacking both sex organs	Spikelet-type prolife-ration
1	58	0	27	1	57	3
2	76	2	29	0	76	3
3	70	14	27	3	63	12
4	89	13	39	1	88	26
5	114	18	41	10	98	33
Total	407	47	163	15	382	77
(%)	(100)	(11.5)	(40.0)	(3.7)	(93.9)	(18.9)

these spikelets, however, a small protuberance differentiated at the site of sexual organs. These protuberances were detected by SEM and found to have similar shape with normal spikelet primordia ; on further development these differentiated to rudimentary glumes, empty glumes, lemma and palea (Figs. 15 and 16). Of the total spikelets, 18.9% developed spikelet-type (S-type) proliferation in which one or more spikelet primordium(a) differentiated instead of normal sexual organs. Figs. 5 to 8, and Figs. 13 to 14 indicate examples of the S-type proliferation viewed by stereo microscope and SEM, respectively. Of the total spikelets, 3.7% developed pistil hyperplasia and have proliferated stigma(ta) or pistil(s). Figs. 2 to 4, and Figs. 11 to 12 show examples of the pistil hyperplasia viewed by stereo microscope and SEM, respectively. The number of blumes, icluding rudimentary glume, empty glume, lemma and palea also changed considerablly ; it increased in 11.5% of the total spikelets, and decreased 40.0% of the spikelets palea. The shape of lemma and palea also changed in about all of the spikelets ; they were lacking in jointing wings between them.

Discussion

The most remarkable morphogenetic change of spikelets investigated in this study was that, above 93% of the total spikelets had ceased to differentiate normal sexual organs. Instead, many of the spikelets developed small protuberance in site of the sexual organs. The shape of this protuberance was identified by SEM to be same shape as that of normal spikelet primordium ; moreover, 18.9% of total spikelets differentiated into well developed S-type proliferation. This indicates that almost all of the spikelets on the straighthead panicle have a potentiality to develop S-type or other -type of proliferation instead of sexual organs. Though frequency of spikelets which have developed pistil hyperplasia were below 4% ; this phenomenon does not simply mean a suppression of growth but it means something of high significance-induction of hyperplasia in pistil morphogenesis. Therefore, it was concluded that the initiation of pistil hyperplasia and S-type proliferation were important characteristics of the morphogenetic change in sexual organs of the straighthead rice plant.

Symptoms of abnormal development in a straighthead rice plants observed by Kitamura[8,9,10] in field surveys were mainly on panicle and floral organs, such as failure in panicle formation, deformed panicles and spikelets, abnormal sexual organs including degeneration of anther, abortion of pollen grains, and malformity of stigma, sterility, and abnormal development of embryo in fertile seeds, which revealed an extraordinarily wide range of variation. The abnormalities reported there were more or less similar to those occurred with a physiological disease in rice plants found in the southern part of U.S.A.[25]. The malformity of stigma and degeneration of anther were similar to that in this study. It is, therefore, considered that pistil hyperplasia and stamen hypoplasia are common characteristics in both types of straightheads—naturally occurred or experimentally induced.

Malformity in spikelets is one of the causes for sterility in rice Floral malformities are induced by environmental disorders, for example, cold weather[15], over flooding[6], high salt concentration[4], high temperature[23], dry weather[1,2]. Genetical factors[3,14,16,18] are also responsible for floral abnormalities. Among the floral malformities induced by these environmental or genetical factors, the pistil hyperplasia and stamen hypoplasia, occurring most frequently and commonly, are of high significance from morphogenetic point of view. Takeoka and his coworkers[16-22] classified the genetically proliferative spikelets into three types—S-, P-, and L-type proliferations. The S-type proliferation has one or more spikelets in it, the P-type proliferation has more than one pistil or a pistil (s) with swollen ovary, and the L-type proliferation has one or more leafy shoot in it. They also observed that these proliferations are not independent of one another, rather they represent three successive stages of the proliferative spikelets, the final stage of which is the L-type. They also observed that a proliferative spikelets, whatever type does it represent is always characterized by any one or more of the following floral organ malformities: (1) no floral organ develops, instead the floral apex grows to produce a spikelet on its head ; (2) the pistil grows normally while the stamens degenerate completely or partially ; (3) there is an increment in the pistil-like organ ; and (4) the spikelet develops into normal shoot with the stamen and pistil being degenerated.

The malformities observed in this study do also resemble to the above mentioned malformities of floral organs. It is therefore concluded that the rice spikelets would show similar types of floral malformities, the pistil hyperplasia and the stamen hypoplasia, in particular, under varied environmental stress.

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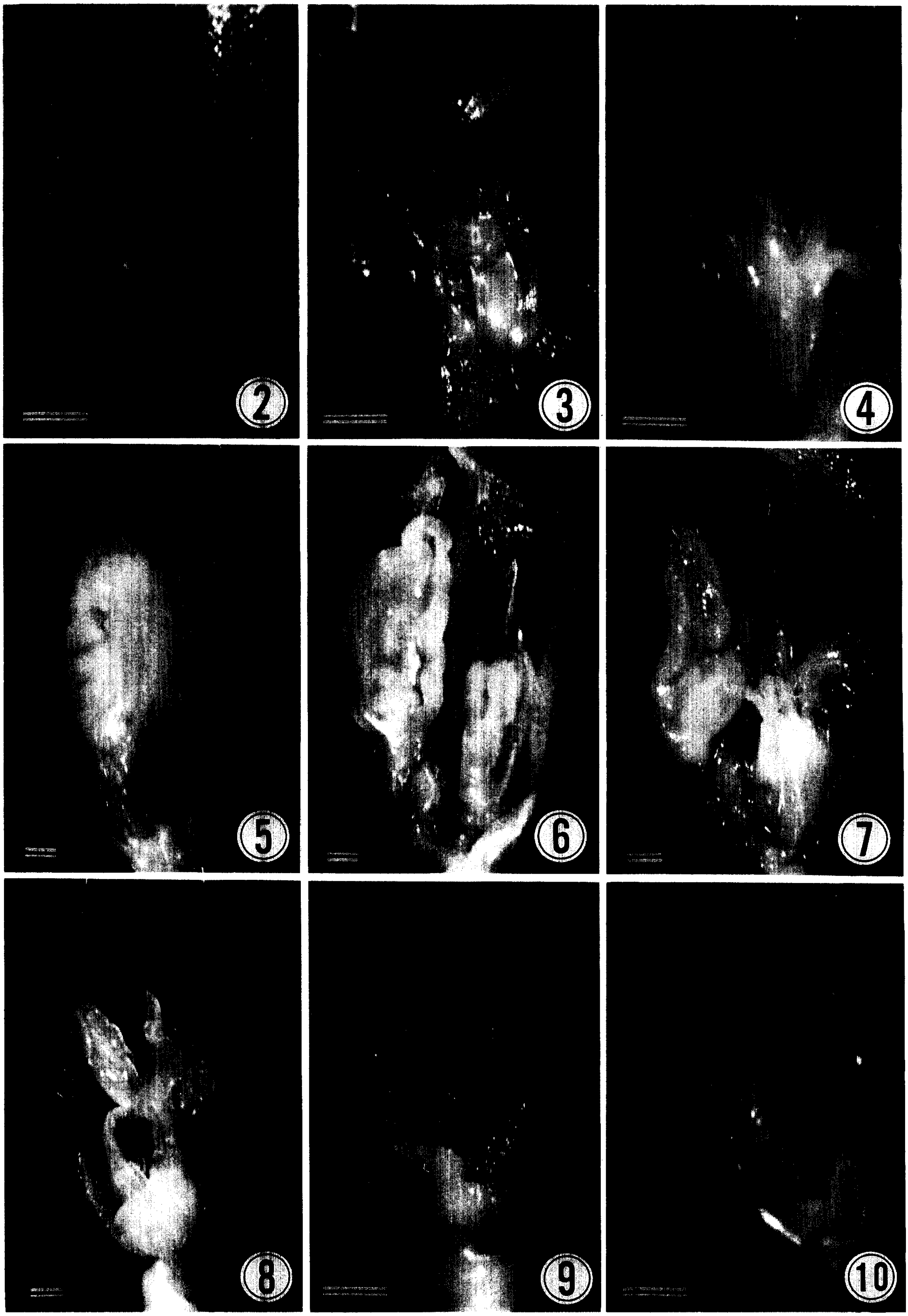
Explanation of figures (Figs. 2—10)

Inside view observed by stereo-microscope of rice spikelets changed morphogenetically due to the induced straighthead ; pistil hyperplasia to have proliferated ovary or stigma (Figs. 2—4, and Figs. 8), spikelet-type proliferation to have repeated spikelet primordium instead of both of ordinary sexual organs (Figs. 5—7) and small protuberance which was identified by SEM to be same as spikelet primordium (Figs. 9—10). Bar in each Fig. 2 to 10 indicates 0.05 cm.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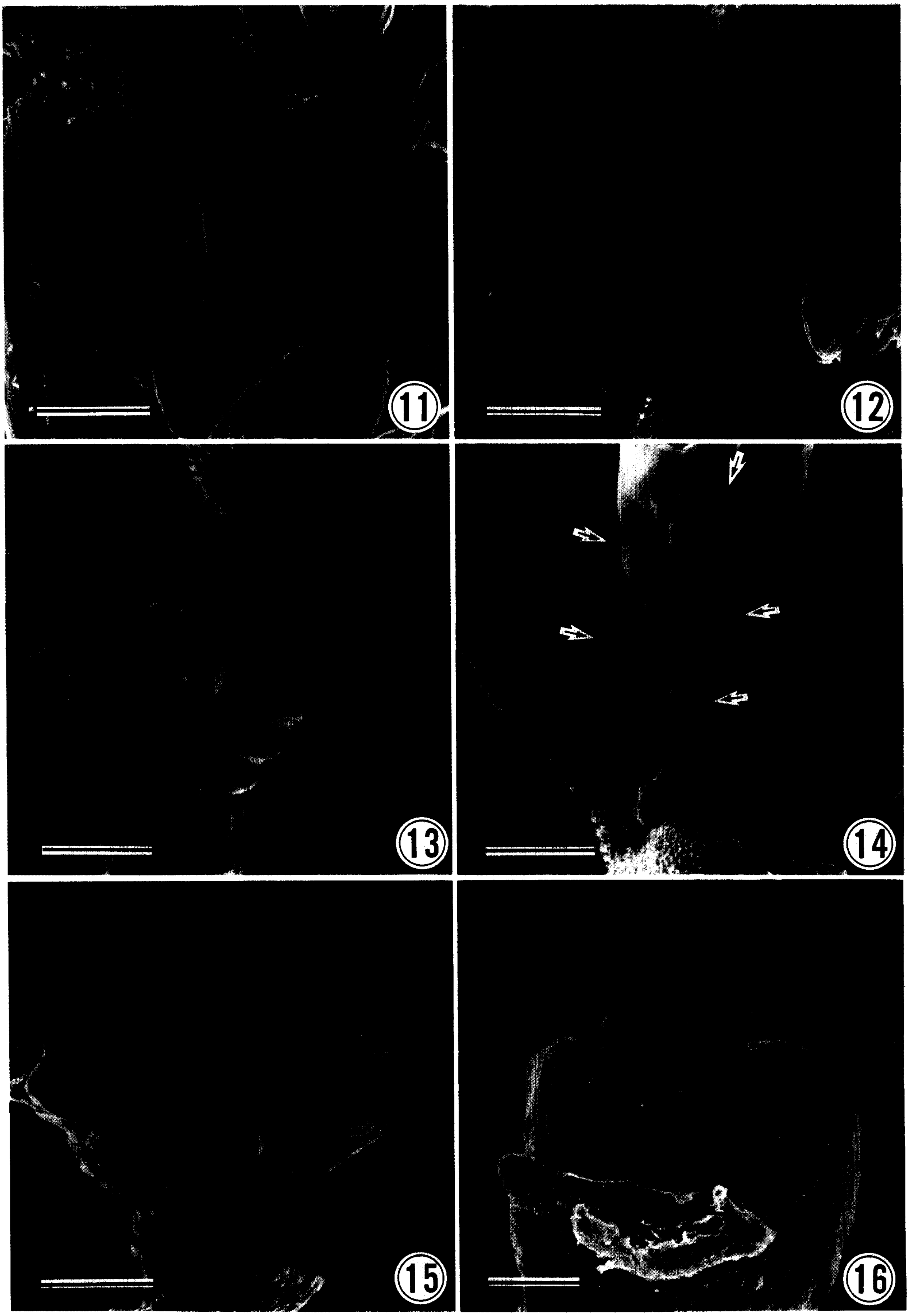
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* In Japanese with English summary.
** Translated from Japanese by the authors.

Explanation of figures (Figs. 11—16)

Side views observed by SEM of inside of rice spikelet changed morphogenetically due to the induced straighthead. Fig. 11 : A case of pistil hyperplasia to have developed one more stigma (arrow). Beside of the pistil a slender glume also has differentiated, base of which similar lodicule. Fig. 12 : Another case of pistil hyperplasia in Which many stigmata differentiated. Fig. 13 : A case of spikelet-type proliferation which two young spikelets are on growth. Fig. 14 : Base of a spikelet-type proliferation to show pedicel with five rudimentary glumes as indicated by arrows. Fig. 15 : Tip of a protuberance having three small spikelet promordia, each of which is at the stage to differentiate rudimentary-, or empty-glume. Fig. 16 : Enlarged view of the spikelet primordium at the stage just after differentiating empty glume. Each bar in Figs. 11 to 15 indicates 0.5 mm, and in Fig. 16 indicates 0.05 mm.