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Morphological Alterations of Flower Induced by Chilling Stress in Rices

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ABSTRACT

Morphological alteration of floral organ development in rice affected by chilling stress was examined. Three varieties of rice were grown under natural conditions and subjected to 12℃ for 3 or 6 days starting from the ineffective tillering stage, before heading stage and returned to natural condition. Headings were delayed by a 6 day chilling treatment. After heading the panicles were collected and examined for any possible alteration in floral organ development. It appears that there were some differences in sensitivity to chilling stress and degree of injury depending on treatment stages and variety. Chuchungbyeo was the most frequent in producing abnormal flowers among the three varieties examined. Meiosis stage was shown to be the most vulnerable to chilling stress in both Chuchungbyeo and Ilpumbyeo and young panicle differentiation stage was the frequent stage to alter flower development in response to chilling stress only in Chuchungbyeo. It was confirmed that abnormalities occurred in pollen due to chilling stress is a major factor leading to low yield, but to some extent the alterations in carpel development may play a certain role in determining a total yield in response to chilling stress at the reproduction stage in rice. There were abnormalities like extra stigmata, extra lemma, double ovary as well as abnormal anther formation in response to chilling stress. Further studies of the phenocopy observed in rice floral development may be useful for an understanding of the resistance against chilling injury during reproductive stages in rice.

Keywords : phenocopy, rice, flower, chilling stress, reproduction.

A flower is one of the most important organs in plants since it has a major role in sexual reproduction. In addition, a flower develops into the major energy sink to deposit photosynthate and finally become a food provider. The correct formation of floral organs in cereal crops is essential to guarantee a maximum agricultural yield. However, it appears that the flower development is very vulnerable to environmental stresses so that the crop yield heavily depends on the environmental conditions and there are many efforts going on to increase resistances to various environment stresses in the areas of crop breeding and plant genetic engineering.

The phenomena of chilling injury on a spikelet of rice and the resulting reduction in yield have been studied intensively. The researches have focused mainly on the abnormality in male organ development and any change in fertility of pollen grains affected by low temperature stress(Ito et al., 1970; Wada et at., 1972; Satake & Hayase, 1974). In addition, there have been many recent reports showing abnormalities in female reproductive organ induced by low temperature stress (Polowick & Sawhney, 1985; Lynch, 1990; Shuff & Thomas, 1993; Takeoka et al., 1993). It appears that both the stamen and the carpel are highly sensitive to low temperatures and low temperature stresses could influence each step of the reproduction process to lead to morphological alterations of flowers and reduce the yields.

The development of a flower is a complicated process orchestrated by several genes that trigger other genes responsible for each step in differentiation of a flower. Some of the genes playing major regulatory roles in flower development have been cloned and analyzed based on molecular studies of homeotic mutations (Coen & Meyerowiyz, 1991; Coen & Carpenter, 1993). However, the more detailed paths in development toward each structure of a flower remain to be researched since the spectrum of the developmental mutation in a flower is very limited due to it's inherent infertility. Therefore, phenomenon of the epigenetic phenocopy that mimic a mutation without an alteration of that genetic materials will provide a good system to study the specific aspects of flower development and to that end, will provide an important clue toward understanding of the interaction between gene expression for flower development and environmental stresses. Further studies will support a strategy for molecular breeding toward crops to resistant low temperature stress.

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MATERIALS AND METHOD

Rice growth and low temperature treatment

The varieties of Dasanbyeo, Chuchungbyeo, and

Ilpumbyeo showing low, medium, and high resistance to chilling stress, respectively(based on information from New Agricultural Techniques, RDA, Korea, 1997) were studied. The germinated seeds were sown in wagner pots on June 10, 1998 and the pots were kept in a submerged condition. The rice plants were grown under natural illumination and temperature conditions before the plants were subjected to a low temperature treatment in 12℃ growth chamber under 16/8 hours(light/dark) condition for either 3 days or 6 days in consecutive manner from the ineffective tillering stage to the heading stage. After the treatment, the plants were returned back to the outdoor condition and cultivated.

Tissue sampling

Three panicles per pot were collected at the time point when the top of the panicle reached the auricle of the flag leaf. Each spikelet with exclusion of the upper five primary rachis-branches from a panicle were dissected and the flower organs were examined under a stereo dissection microscope. If necessary, the spikelets were fixed in 10 ml of the FAA solution (50% ethanol, 5% glacial acetic acid, 3.7% formaldehyde; McFadden et al., 1998) for 15 min under vacuum-infiltration. The fixed samples were washed with 50% ethanol and stored.

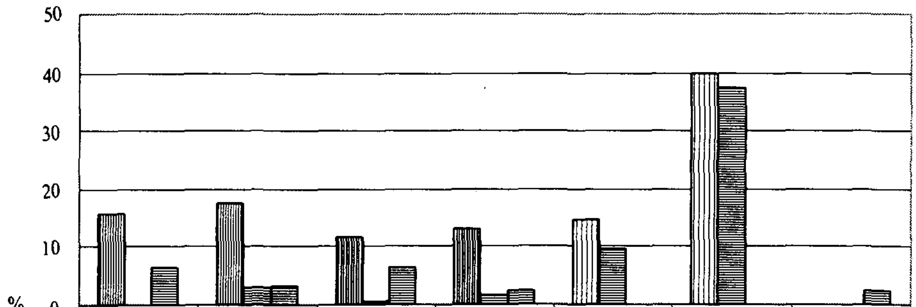
RESULTS AND DISCUSSION

The effects of chilling stress on general growth of rice plants

In general, the 6 day treatments of chilling stress made the plant growth and heading slower than the 3 day treatments which is consistant with the results of Kabaki et al.(1982). Headings were delayed for 2, 4, and 10 days by chilling treatment for 6 days in Chuchungbyeo, Ilpumbyeo, and Dasanbyeo respectively. The three day cold treatment also showed delayed headings to some extent in Ilpumbyeo and Dasanbyeo but no difference in Chuchungbyeo. Choi & Oh(1976) had reported 2-6 days delay of heading in different varieties of rice due to chilling stress. There were also differences in degree of the injury as an effect of chilling stress among the varieties such that Chuchungbyeo and Ilpumbyeo were better than Dasanbyeo.

The abnormalities in rice flower induced by chilling stress

The average frequencies of morphological abnormalities induced by chilling stress during early reproductive stage were 5, 5.3 and 14% in Ilpumbye, Dasanbye, and Chuchungbye, respectively and the extents of the frequency varies depending on the time points of the treatment(Fig. 1). Chuchungbyeo showed the highest frequency of morphological abnormality especially in pollen structures. The Dasanbyeo known to be the most sensitive to chilling stress among the three varieties showed the lowest degree of frequency in abnormality especially on the stigma. The presence of one or two additional stigmata was the most frequent phenocopy observed and it may draw more interest as a suitable system for anatomical and molecular study of the development of a multiple stigma. In case of Ilpumbyeo, pollen immaturity may be a major defect to be induced by chilling stress. Besides, there were some other unique abnormalities observed, though at a rare frequency.



	IETL	YPDF	PSRD	SPDF	MEIO	FULL	CONT
CHUCHUNG	15.8	17.5	11.6	12.9	14.7	40	0
ILPUM	0	2.9	0.6	1.5	9.6	37.5	0
DASAN	6.4	3.3	6.4	2.5	0	0	2.5

Fig. 1. The frequency(%) of the flower phenocopies induced by chilling stress at different stages of flower development in three varieties of rice. (IETL: ineffective tillering stage, YPDF: young panicle differentiation stage, PSRD: primary and secondary rachis branch differentiation stage, SPDF: spikelet differentiation stage, MEIO: meiosis stage, FULL: continuous chilling treatment, CONT: no chilling treatment).

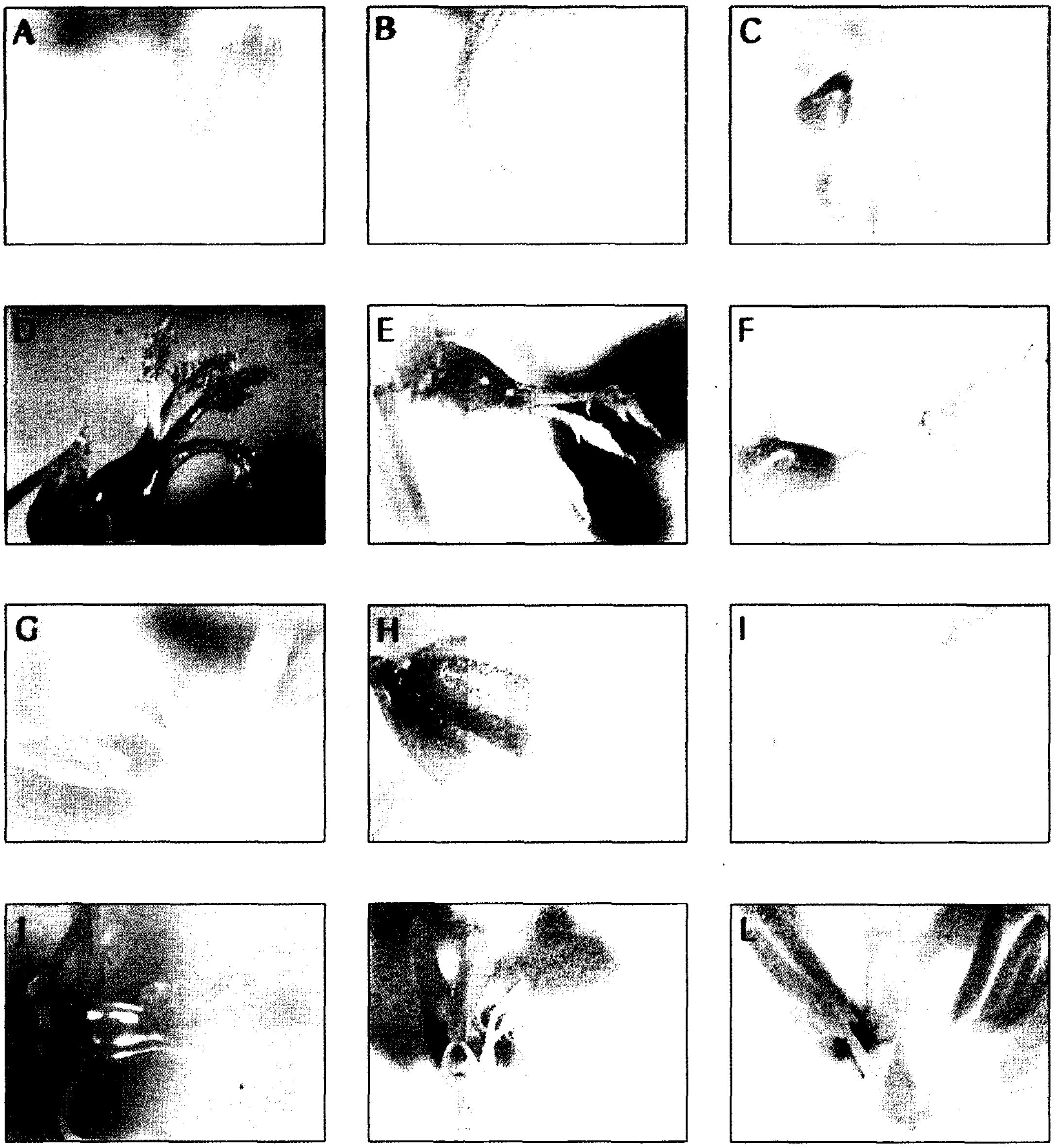


Fig. 2. The types of flower phenocopy induced by chilling stress during flower development in rice A: one abnormal anther, B: different sized anther, C: malformed anther, D: four-stigmata, E: three- stigmata, F: an appendage between stigmata, G: a premature extra stigmata) H: an extra lemma, I: double ovary, J: a malformed tissue between stigmata, K: carpel-like tissue in filament, L: a fusion of two filaments.

Some spikelets were observed with an additional lemma or with double ovaries(Fig. 2-H).

Rice plants grown under a prolonged treatment of chilling stress showed the highest frequency of morphological abnormalities except Dasanbyeo which was completely dead completely at the day of heading (Figure 1-FULL). Without the chilling treatment, Dasanbyeo showed a 2.5% of frequency for morphological abnormality, which may indicate the degree of frequency of phenocopy occurred spontaneously or in response to a possible chilling stress under natural conditions(Fig. 1-CONT).

The flower phenocopy could be classified into several groups depending on the tissue in that an abnormality occurred as reported in rice and in tomato by other researchers (Takeoka et al., 1993; Lozano et al., 1998). An appendage in between two stigmata is one of the major abnormalities found and it appears that the structure may be a primordia for another set of stigmata since many other phenocopies

Table 1. Types and frequencies(%) of phenocopies developed in response to chilling stress among the three different varieties of rice.

Site	Description	Chuchung	Ilpum	Dasan	Total
Stamen	malformed anther(A†, C)	37.3	9.6	1.43	48.3
	immature anther	5.1	15.3		20.4
	extra tissue in anther	0.1	1.0	0.6	1.7
	abnormal size(B)	0.1	5.4		5.5
Carpel	multiple stigmas(D, E, G)	0.3		7.1	7.4
	appendage between stigmata(F, J)	0.9		7.2	8.1
	malformed ovary	1.4	0.6	1.0	3.0
	double ovary(I)	0.65	0.65		1.3
Others	malformed tissue (K, L)	1.2	0.8	1.0	3.0
	extra lemma(H)	0.3	1.0		1.3

†: A to L in parenthesis correspond to the number in Fig. 1.

showed a set of small or full-sized stigmata at the site of the appendage(Fig. 2-D-G, J). There were also two types of abnormalities found in the stamen of either an unbalanced structure of pollen due to an asymmetric growth or a short filament (Fig. 2-A, C). An ovary could be a target for abnormality, that is, wrinkled shaped ovary, or an ovary with a cleft on the surface, and some ovaries showed an ovaries exposed out of the cleft. In addition, flowers with double carpels or 9 sets of pistils were observed.

Flower phenocopy and developmental stage

The developmental stages for each of the flower phenocopy which occurred in three rice varieties are listed in Table 1. There was no direct correlation between the types of phenocopy and developmental stages. Even though there was no direct trend showing any correlation between types of phenocopy and developmental stages, it may be possible to draw some conclusions on the trends. First, it appears that the stage of young panicle differentiation is so sensitive in many directions of development to chilling stress, in the light of that almost all variety of abnormalities developed. Second, the ineffective tillering stage was shown to be the most vulnerable in terms of frequency for phenocopies occurred in both Chuchung- byeo and Dasanbyeo but not so significant in Ilpumbyeo. Third, each rice variety seems to have its rather favored stage for a specific type of phenocopy occurrence since there were subtle differences in frequency of phenocopy at each of the developmental stages. Both Chuchungbyeo and Ilpumbyeo showed a medium degree of phenocopy frequency due to chilling stress at the meiosis stage but Dasanbyeo did not show any phenocopy. However only Dasanbyeo showed a high degree of phenocopy frequency by a chilling treatment at the stages of primary and secondary rachis branch differentiation.

In conclusion, the meiosis stage is the most vulnerable in flower development, as well as for a maximum level of yield that is corresponding to what many other researchers had reported (Wada et al., 1972; Oh, 1981). However Dasanbyeo seems to be an exception and it seems like that the genetic background from an indica side may be responsible for that exceptional response but the exact reason is not known yet. Also it appears that the chilling stress at other than meiosis stage during the early reproductive period may affect flower development and yield and affect development of floral organs other than pollen. Therefore it may be concluded that an overall reduction in yield may result from not only the defects in pollen development but also the abnormal development of the carpel.

The phenocopy phenomenon is epigenetic that is induced by abnormal control in expression of normal genes due to environmental cues. Therefore it will provide a good experimental system to study flower development as well as the interaction between a developmental program and environmental stress. In addition, the practical interest on elucidation and development of resistance against chilling injury in rice plant can be a good reason to emphasize further studies on phenocopy in rice.

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