Floral morphology in rice grown under cold temperatures at booting and flowering and its effect on spikelet sterility

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Abstract

Booting and flowering in rice (Oryza sativa L.) are considered to be the two most sensitive stages to cold temperature stress. The controlled temperature glasshouse experiment compared a population of 120 genotypes from F6 Kyeema//Kyeema/NorinPL8 (KKN) when exposed to cold air temperature at the booting and flowering stages. The study aimed to examine the relationship between percentage of spikelet sterility (SS) and floral characteristics, namely the number of dehisced anthers (NoDA), anther dehiscence length (ADL), the number of pollen grains on stigma (PoS) and the anther length (AL) when subjected to cold temperature at the two development stages. Two sets of genotypes were sown 18 days apart and grown at 28/21°C day/night controlled temperature glasshouse, and were moved to the cold room (21/15°C day/night) at heading (set-1) and at early booting stage (set-2). A highly significant genotypic difference existed in percentage SS in both flowering (ranged from 49-100%) and early booting (4-99%) with flowering stage having higher average SS (88 vs 57%). A significant positive association existed between SS of flowering and booting (r=0.39**) with five genotypes performing consistently well. Highly significant negative correlations existed in both flowering and booting stage between SS and all the floral traits measured. Furthermore, multiple regression analysis indicated that 37% of the variation in SS was explained by the number of dehisced anther, anther dehiscence length and anther length when exposed to cold at flowering stage, while at booting stage the number of dehisced anther and anther length alone explained 58% of the variation in SS. The importance of the number of dehisced anther in explaining variation in SS has been highlighted and plays a significant role in cold tolerance.

Key words

Spikelet sterility, pollen, dehiscence, flowering, booting.

Introduction

The most sensitive stage of cold stress in rice is the booting stage, particularly the early pollen microspore stage (Satake and Hayase, 1970) followed by the flowering stage (Matsuo et al, 1995). Cold temperature during the two most sensitive stages directly affects spikelet sterility and leads to low grain yield. Meiosis which takes place at booting is susceptible to cold temperature and therefore affects pollen development (Matsushima, 1966). The early pollen microspore stage usually occurs 10 to 12 days prior to heading (Satake, 1976). Just after heading, flowering occurs and can take up to 5-7 days for all spikelets within a panicle to complete(Suzuki, 1982). Most spikelets flower in the morning, starting about 9am to 12:00 on a sunny day (Suzuki, 1982). Cold tolerance is often measured by spikelet sterility and is largely the result of damage of floral components leading to failure of fertilization (Nishiyama, 1984)

Matsui and Omasa (2002) under high temperature stress at flowering concluded that the reduction in the number of fertile engorged pollen in anther was the main cause for spikelet sterility. Fewer engorged pollen in anther led to less pollen swelling which resulted in anther indehiscence. Furthermore, under heat stress, Matsui et al (1999) reported that poor anthers dehiscence lead to reduced pollination and caused higher spikelet sterility and reduced grain yield. Matsui and Kagata (2003) examined that under heat stress the number of pollen grains deposited on the stigma was not influenced by the length of anther (ranged from 1.46 to 2.52 mm) and the length of apical dehiscence but was significantly correlated with the length of basal dehiscence.

This experiment was conducted to evaluate the cold tolerance of 120 genotypes (including 2 parents (Kyeema and NorinPL8) and Sherpa) at the booting and flowering stages, and to explore the floral characters contributing to spikelet sterility. The objectives of the experiment were (1) to determine genotypic variation in spikelet sterility within a population 120 genotypes of F6 KKN exposed to cold stress at the booting and flowering stages, (2)) to identify floral characters that are contributing to cold tolerance at the booting and

flowering stages, (3) to identify the cold tolerant genotypes at booting and flowering stage based on percentage of spikelet sterility, (4) to compare the consistency of line performance in the booting and flowering stages of the 120 genotypes.

Materials and methods

Two sets of the 120 genotypes were sown 18 days apart and grown under warm conditions (27/23^{o}C day/night. The first planted set were moved to the cold temperature glasshouse 18/14^{o}C at heading while the second planting at early booting stage for 14 days. Tube pots (5x5 cm and height 12 cm were filled with Gatton Black vertosol soil up to 1 cm from top of the pot and 1.0 g of slow release fertilizer (Osmocote plus 3-4 months: 16N–9P–12K) provided to ensure no nutrient limitation. Each tube was placed inside a second tube pot with 1.5cm cement layer in the base to reduce root escape. 5 seeds of each line were manually sown to a depth of 1.5 cm. Seedlings were thinned to leave 2 seedlings per tube at 11 days after sowing (DAS). This was then thinned to 1 seedling per tube at 15 DAS. Tubes were held in stable position within a wire mesh inside a water tank. Water level was gradually increased by 3cm every four days reach a final depth of 4cm above the soil surface with this flooded condition maintained for the duration of the experiment. During the growth period, the tubes were rotated weekly within replication to minimize any potential local influences including light and temperature.

Set-1 Flowering stage, on average 12 days after moving into the cold temperature glasshouse, 6 spikelets on the main stem panicle were randomly sampled shortly after floret opening , and from fixed positions the 3rd-5th spikelet at the 1st-5th upper branches for the booting stage cold (Set-2). Fresh spikelets were placed immediately into an empty petri dish, and stored at room-temperature. Sample processing was the same for both Set-1 and Set-2 with 3 spikelets out of 6 dissected. From each spikelet 6 anthers were examined for dehiscence and counted, 3 anthers randomly selected (from 6) were measured for anther length and anther dehiscence length under a stereo microscope using a digital micrometre. From the same spikelet, stigma was dissected and pollen on stigma counted. Stigma was stained with Iodine-potassium iodide solution (IKI; 0.5 g I2 and 2 g KI in 100 mL of H2). All data was analysed using Genstat 16th edition with genotype means compared using least significant difference (LSD 5%).

Results and discussion

SS induced by cold temperature at flowering stage was high (88%) and ranged from 49 to 100%, while at booting SS averaged 57% and ranged from 4 to 100%. A highly significant ($p<0.01$**) genotypic difference existed in SS and all floral traits measured at both development stages (Table 1).

Table 1. Percentage of spikelet sterility (SS), anther dehiscence length (ADL; µm), number of pollen grains on stigma (PoS), anther length (AL); and number of dehisced anther (NoDA); the minimum, maximum and mean value of the 120 genotypes at flowering (F) and booting (B) stages.

Genotypes	SS		Anther dehiscence length **(µm)**		The number of pollen on stigma		Anther length **(µm)**		The number of dehisced anther	
	F	B	F	B	F	B	F	B	F	B
Min	49	4	0	0	0	0	1056	704	0.0	0.0
Max	100	99	1237	1211	228	194	2168	2382	6.0	6.0
Mean	88	57	442	604	45	48	1747	1135	2.1	3.5
LSD (5%)	23**	29**	475**	468**	77**	80**	139**	479**	2.5**	2.7**
CV (%)	16	31	67	48	105	104	5	26	73.3	48.5
Cultivars										
Norin	86	4	857	856	93	52	1838	1219	5.8	5.8
Kyeema	97	56	206	362	28	3	1740	1080	2.0	2.0
Sherpa	79	30	951	577	214	52	1717	1287	2.5	2.5

Under flowering cold stress, Sherpa and Norin which are known to be cold tolerant cultivars had relatively high sterility at 79% and 86% (NSW Department Primary Industry, 2012; Saito et al, 2001). Compared to Sherpa, 21 genotypes had lower SS with the lowest SS achieved by line 12-223 (49%). Kyeema had 97% SS. The 20 most tolerant/susceptible genotypes had an average of 63% SS and 99% SS respectively. NorinPL8 was confirmed as the most tolerant cultivar at the booting stage which had only 4% SS while Sherpa and Kyeema had 30% and 56% SS respectively. A significant positive correlation existed between SS of genotypes exposed to cold at flowering and booting (r=0.39**) with Five genotypes performing consistently well (9-220, 9-229, 10-227, 10-230 and 11-227).

Anther dehiscence length (the sum of basal and apical dehiscence) ranged from 0 to 1237µm at flowering stage stress. Among the 3 cultivars, Sherpa and NorinPL8 had the longest anther dehiscence length at 951 and 857µm respectively, while Kyeema had the shortest dehiscence at 206 µm. There were 5 genotypes which had longer anther dehiscence length than Sherpa when dehiscence occurred under cold temperature conditions, 10-218 (1236 µm); 15-215 (1093 µm); 9-220 (1021 µm); 9-216 (1000 µm) and 10-230 (974 µm). In contrast, there were 6 genotypes which had no dehiscence at all (11-226, 11-229, 14-221, 13-220, 15-218 and 15-217). In flowering stage, dehiscence occurred cold exposure, however, the development of the panicle including pollen differentiation and development had all occurred under warm conditions, therefore the number of pollen in anther should have been able to reach genetic potential. The number of pollen in anther has been shown to be positively associated with pollen swelling which is hypothesised to be the driving force behind anther dehiscence (Matsui and Kagata, 2003). However, in the current experiment, with ample pollen produced under warm conditions the cause of indehiscence is likely to be the pollen swelling process itself was impaired. Indehisced anthers resulted in zero pollen grains deposited on the stigma.

At booting when dehiscence occurred under warm conditions after exposure to cold, NorinPL8 (856 µm) and Sherpa (577 µm) had longer dehiscence than Kyeema (362 µm). There were 25 genotypes which had significantly longer dehiscence than NorinPL8, ranging from 861-1211 µm. As a result of cold exposure at the booting stage 6 genotypes (different to those at flowering stage) had no anther dehiscence (10-218, 11-217, 11-219, 12-218, 12-220, 14-226).

The longer dehiscence the higher the possibility for pollen grains to be shed and intercepted by the stigma. Satake and Yoshida (1978) suggest that it is necessary to have 10 germinated pollen grains on the stigma to ensure successful fertilization. However, Matsui and Kagata (2003) examining rice under heat stress discovered that only 50% of the number of pollen grains on stigma germinate, therefore at least 20 pollens on stigma are necessary for successful fertilization, with 40 pollens on stigma considered as sufficient and 80 pollens on stigma as ample to ensure fertilisation. A similar result was found by Jagadish et al (2010) who revealed variation in spikelet sterility in heat stress at flowering stage was highly correlated with the proportion of spikelets with more than 20 germinated pollen grain on stigma. While in this experiment, the majority of genotypes a range of dehisce occurred and the minimum anther dehiscence length under flowering-cold-stress to ensure shedding of 40 pollen on stigma was 400µm, under booting-cold-stress this was 525µm.

In flowering-cold-stress, among the 3 cultivars, Sherpa produced the highest number of pollen on stigma (214). There was only one genotype, 9-216 which had higher number pollen on stigma than Sherpa. However 13% of genotypes had ample number of PoS (≥ 80 pollens) which should have sufficient pollen grains on stigma to maintain low SS%. While 66 of genotypes or 59% of genotypes under flowering cold had less than 40 pollens deposited on stigma, or higher possibility of unsuccessful fertilization.

In booting, 18% of the total genotypes had more than 80 pollens grains on stigma, with the highest number achieved by9-226 (194 pollens on stigma). While 32% of genotypes had more than 40 pollen grains on stigma and 50% of the population was considered to have sufficient pollen grains on stigma to ensure successful fertilization. However, 50% of genotypes had less than 40 pollen grains on stigma with 6% of genotypes having no pollen grains deposited on stigma. Anther dehiscence length had a strong positive correlation to the number of pollen grain on stigma both in flowering (r=0.82**) and in booting (r=0.68**).

When exposed to cold temperature, anther length was the floral trait with the largest difference between the two development stages. While Suzuki (1982) suggested AL to be important for cold tolerance Matsui and Kagata (2003) found that the number of pollen grains deposited on the stigma was not influenced by the AL which varied from 1.46 to 2.52 mm, but that the length of basal dehiscence (ranged from 0.26 to 0.58 mm) was significantly correlated to PoS.

When exposed to cold at flowering anther development was already completed and consequently anther length was unaffected by cold temperature stress and had an average of 1747 µm (1056-2168µm) while at booting stage cold stress effected anther elongation and resulted in a significantly reduced (by 36%) the anther length of 1135µm (704-2382) µm. This result supported the result of Suzuki (1982). The mean of anther length was greater at the booting stage indicating the adverse effect of booting stage cold on anther elongation.

Highly significant negative correlations existed in both flowering and booting stage between SS and all the floral traits measured (Table 2). The decrease in anther length (AL) at booting may lead to poor anther

dehiscence, however, in this experiment at booting-stage, the greater number of dehisced anthers may have compensated and resulted in sufficient number of pollen grains on stigma. The number of dehisced anthers at booting explained 56% of the variation in SS while at flowering stage the number of dehisced anther (NoDA) explained 33% of variation.

Furthermore, multiple regression analysis indicated that 37% of the variation in SS was explained by the number of dehisced anther (NoDA), the anther dehiscence length (ADL), and the anther length (AL) when exposed to cold at flowering stage, while at booting stage the number of dehisced anther (NoDA) and the anther length (AL) alone explained 58% of the variation in SS.

Table 2. Variation of SS in correlation to floral traits at flowering and booting.

Variables	F	B
DTH	0.41**	0.18ns
ADL (um)	-0.47**	-0.70**
Basal DL	-0.36**	-0.58**
Apical DL	-0.54**	-0.46**
PoS	-0.51**	-0.53**
AL (um)	-0.41**	-0.38**
No Dehisced Anther	-0.57**	-0.75**

Conclusion

Within the KKN population, all floral characteristics (anther dehiscence lenth, the number of pollen grains on stigma, anther length and the number of dehisced anther) had strong negative correlation to SS, both in Flowering and booting stages. The decreased AL at booting stage indicated the adverse effect of booting stage cold on anther elongation. The importance of the number of dehisced anther in explaining the total variation in SS, particularly at the booting stage was highlighted and plays a significant role in cold tolerance.

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