Effect of Temperature on Pollen Fertility in Inter-Subspecific Rice Hybrids

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Abstract: Seventeen rice varieties and hybrids of different types (indica, japonica, javanica, indica hybrid, japonica hybrid and inter-subspecific hybrid) were evaluated to determine the effect of temperature on pollen fertility in inter-subspecific hybrids. The pollen fertility of inter-subspecific hybrids was greatly reduced when average daily temperature dropped to 22.0 – 23.0°C at meiosis stage, and the extent of pollen fertility reduction varied greatly with respect to different hybrids. However, the pollen fertility reduction of indica and japonica hybrids and conventional varieties was not obvious under the same regime of temperature conditions. When the average daily temperature dropped to 20°C, the pollen development of conventional varieties and hybrids was also affected. Correlation analysis revealed that there existed a positive correlation between pollen fertility and average daily temperature. A significant difference (P<0.01) was also found between the two correlation coefficients i.e. inter-subspecific hybrids and conventional varieties. Temperature at meiosis stage of pollen mother cell was a key factor in pollen developing, and the pollen fertility of inter-subspecific hybrids was more sensitive to low temperature than that of traditional variety.

Key words: hybrid rice; temperature; pollen fertility; inter-subspecific hybrid

The strong heterosis in the cross between indica (Oryza sativa subsp. indica) and japonica (O. sativa subsp. japonica) rice has been studied in recent decades aiming at exploiting such heterosis to develop the indica-japonica hybrids which have promisingly significant yield advantage over intra-subspecific hybrids. Many biological constraints have been experienced as a consequence of the extensive genetic differentiation between indica and japonica rice. The first one is the poor fertility of the hybrids in the majority of indica-japonica crosses, though it has now been at least partly overcome by employing wide-compatible (WC) varieties that are capable to produce fertile hybrids when crossed to both indica and japonica varieties [1, 2]. It has been shown that wide compatibility is conditioned by an allele (ŚF) at the Ś locus located on chromosome 6. However, the fertility of many indica-japonica hybrids is very sensitive to temperature fluctuation, which is another important problem needed to be assessed. It has been observed that hybrids containing the Ś allele have normal fertility under high-temperature conditions, but the fertility is greatly reduced under somewhat lower temperature which does not affect the fertility of the parents. It has been shown that an average daily temperature falling below 24°C at booting stage, can result in a significant fertility reduction in some indica-japonica hybrids, and the genetic analysis suggested that the low temperature sensitive sterility was controlled by two or three QTLs, independent of occurrence of wide compatibility genes [3-5].

Previous studies reported that there exist correlation between spikelet fertility and pollen fertility, and there are at least seven alleles which govern the pollen fertility in indica-japonica hybrids [6-15]. However, little study on the pollen fertility of indica-japonica hybrids affected by low temperature has been conducted. The main objectives of the present study are to determine how does the temperature at the booting stage affect the pollen fertility in inter-subspecific rice hybrids. It is believed that an accelerated understanding of the problem should provide a basis for understanding the genetic mechanism of low-temperature-sensitive sterility, and thus facilitate the utilization of indica-japonica heterosis.

MATERIALS AND METHODS

The materials used in this study included six inter-subspecific hybrids, one indica hybrid, one japonica hybrid, and nine conventional varieties (Table 1). All the F₁ hybrids and their parents were planted on 10th May, 25th May, 10th June, 25th June and 10th July, 2001 during rice-growing season, at the experimental
farm of the Jiangsu Academy of Agricultural Sciences, Nanjing, China. Field management was carried out following normal agronomic practices. The heading time of each hybrid and parent was recorded. The 10-15th day before heading was referred to as the meiosis stage of pollen mother cell. The data of weather were provided by the Weather Research Group, Institute of Agricultural Modernization, Jiangsu Academy of Agricultural Sciences.

Anthers were collected from spikelets just before flowering. Pollen grains taken out from anthers were suspended in a potassium iodide solution (KI-I2). Pollens with normal spherical shape and dark blue color were referred to as the fertile, otherwise as the sterile. Number of normal and sterile pollen grains were counted under microscope. Pollen fertility was defined as the normal pollen grains percentage of the total pollen grains.

RESULTS

Correlation coefficient analysis between pollen fertility and temperature

The positive correlation between pollen fertility and temperature at meiosis stage of pollen mother cell for all tested materials is indicated in Table 2. The average daily temperature and daily low temperature are the ideal indices to detect the effect of temperature on pollen fertility. The remarkable difference was also found between r12 and r21 in the comparison of which the t value (3.1, >t0.01=2.58) was obtained by converting r into Z, suggesting pollen fertility of inter-subspecific hybrid was more suffered from the low temperature than that of conventional variety.

**Effect of temperature on the pollen fertility of conventional rice varieties**

Under normal temperature conditions, all tested varieties displayed normal pollen fertility from 86.3% to 99.9%. However, for the conventional rice varieties that heading during the first ten days of October, when the average daily temperature at the meiosis stage of pollen mother cell dropped to 22.1 - 23.5°C, the pollen fertility was reduced to 48.9% - 88.1%. The pollen fertility of Takanari (*indica*) and Dular (*javanica*), which heading during the last ten days of October, plummeted to 1.7% and 7.6%, respectively, when the average daily temperature declined to 19.4°C at the meiosis stage, whereas the pollen fertilities of other rice varieties were often in the intermediate range between 20% and 60% (Fig. 1-A, B, C, D). This suggested that the daily mean temperature less than 19°C at the meiosis stage of pollen mother cell had larger influence on pollen fertility of conventional rice variety, and the pollen fertility of japonica variety was higher than that of indica one under the same low temperature conditions.

**Effect of temperature on the pollen fertility of intra-subspecific hybrids**

For two intra-subspecific hybrids, the indica hybrid Yueyou 938 derived from Honglian type Yuetai A and R938, the japonica hybrid 86 you 8 from BT type 863 A and R8, the pollen fertility was 49.6% and 48.8%, respectively under normal temperature conditions. When the temperature dropped to below 23°C at the booting stage, the pollen fertilities of the two hybrids were about 45% (Fig. 1-D). However, when the daily temperature approached to below 18.8°C, the pollen fertility of Yueyou 938 plummeted to 7.1%. Yuetai A and 863 A were characterized as gametophytic sterile lines, the F1's theoretical pollen fertilities could be at 50%. Therefore, the daily mean temperature less than 22.5°C at the meiosis stage of pollen mother cells seldom had any effect on the pollen fertility of intra-subspecific hybrids Yueyou 938 and 86 you 8. However, along with the decrease in temperature, the pollen fertility of Yueyou

### Table 1. Rice type of the tested hybrids and varieties.

<table>
<thead>
<tr>
<th>Variety or hybrid</th>
<th>Name of tested materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-subspecific hybrid</td>
<td>3037/02428, Yunleng 10 / Takanari, IR36/Dular, Nikken 2/IR36, Peiai 64S/9311, Peiai 64S/E32</td>
</tr>
<tr>
<td>indica hybrid</td>
<td>Yueyou 938</td>
</tr>
<tr>
<td>japonica hybrid</td>
<td>86 you 8</td>
</tr>
<tr>
<td>javanica variety</td>
<td>Dular</td>
</tr>
<tr>
<td>indica variety</td>
<td>Yangluxian 2, Takanari, IR36, Teqing, 3037</td>
</tr>
<tr>
<td>japonica variety</td>
<td>02428, Nikken 2, Yunleng 10</td>
</tr>
</tbody>
</table>

### Table 2. Correlation coefficients between pollen fertility and temperature at the meiosis stage of pollen mother cell.

<table>
<thead>
<tr>
<th>Temperature parameter</th>
<th>Inter-subspecific hybrid</th>
<th>Variety</th>
<th>indica or japonica hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Average daily temperature</td>
<td>0.9215** (r12, n=18)</td>
<td>0.6546** (r21, n=48)</td>
<td>0.7575* (r12, n=10)</td>
</tr>
<tr>
<td>Average daily high temperature</td>
<td>0.8744** (r12, n=18)</td>
<td>0.5914** (r21, n=48)</td>
<td>0.6920* (r12, n=10)</td>
</tr>
<tr>
<td>Average daily low temperature</td>
<td>0.9034** (r12, n=18)</td>
<td>0.6543** (r21, n=48)</td>
<td>0.7744** (r12, n=10)</td>
</tr>
</tbody>
</table>

**,**Significant at 5% and 1% levels, respectively.

*The pollen fertility of four typical inter-subspecific hybrids was used to calculate the correlation coefficients.
938 heading on 24th October dropped to 7.1% when the daily mean temperature and low temperature were 18.8 and 14.5°C, respectively at the meiosis stage of pollen mother cell, indicating that the mean temperature under 19°C at booting stage could cause serious harm to pollen development of indica hybrid rice Yueyou 938 and 86 you 8.

**Effect of temperature on the pollen fertility of inter-subspecific rice hybrids**

A noticeable reduction of pollen fertility of the four typical inter-subspecific rice hybrids was observed at the booting stage when the daily mean temperature dropped to below 22.5°C, while it had negligible effect on the pollen fertility of their parents (Fig. 1-A, B, C, D). For
example, when the daily mean temperature came down to 22.2°C, the pollen fertility of 3037 and 02428 was 68.3%, 89.8%, respectively, while their F₁’s pollen fertility dropped to 13.1%. Previous study demonstrated that japonica variety “Yunleng 10” harbors genes for cold tolerance at germinating stage[16, 17], the low temperature has almost no effect on its pollen fertility when the daily mean temperature declined to 19.2°C, but the F₁’s pollen fertility was greatly reduced to 13.4% under the same temperature conditions.

The two renowned crosses, Peiai 64S/9311 and Peiai 64S/E32, had higher pollen fertility as compared with other inter-subspecific rice hybrids when the daily mean temperature dropped to below 19.2°C, the pollen fertility of Peiai 64S/E32 being as high as 42.3% at 19.2°C. Thus, there existed considerable difference of low temperature sensitivity among inter-subspecific rice hybrids.

**DISCUSSION**

During panicle initiation, meiosis of pollen mother cell or flowering stages, the rice plant is more sensitive to low temperature, especially in the meiosis stage of pollen mother cell[18, 19]. Therefore, in this report, we focused on the effects of the average daily temperature, average daily high temperature and average daily low temperature on pollen fertility.

Low-temperature-sensitive sterility has become one of the major obstacles for the utilization of inter-subspecific heterosis in rice. When the daily mean temperature dropped to 22-23°C at the booting stage, the fertility of the typical inter-subspecific hybrid 3037/02428 was greatly reduced [3], and the pollen abortion was the main cause of sterility, similar to the results of cold injury in conventional varieties [20]. Our results showed that the pollen fertility of inter-subspecific hybrids is more sensitive to low-temperature than that of intra-subspecific hybrids and conventional varieties, and that the extent of pollen sterility reduction varies greatly among different inter-subspecific hybrids depending on the genotypes of the parents. Thus large scale screening and testing for temperature-insensitive germplasm would be needed to broaden the germplasm basis for the development of productive inter-subspecific hybrids.

Japonica variety “Yunleng 10” was identified as the cold tolerant rice variety during germinating stage by the research in the Yunnan Academy of Agricultural Sciences, Kunming, China. Our results showed that relatively low temperature has little effect on the pollen fertility of Yunleng 10, but the F₁’s pollen fertility of Yunleng 10/Takanari is greatly reduced under the same temperature conditions. Thus further research will be needed to ascertain the possibility of its utilization in rice breeding program and make clear the genetic basis of cold tolerance.

**REFERENCES**


