Can Hot-Water Emasculation Be Applied to Artificial Hybridization of Indica–Type Cambodian Rice?

Ly Tong and Tomohiko Yoshida

(¹United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology; ²Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan)

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In rice breeding, clipping and vacuuming (Coffman et al., 1980) are commonly used to remove pollens in IRRI and U.S.A.; both of methods require more labor and need a highly skillful operation. Hot-water emasculation is a method popularly used in japonica rice breeding programs (Kondou, 1939; Matsubayashi et al., 1965), deactivating pollen by hot water. It is easy to handle for both the skillful person and the unskillful person and requires less labor. It is not known why breeders other than Japanese are not willing to accept this hot-water emasculation method, but one reason might be the absence of reports about this method applied to indica cultivars. The purpose of this study is to apply the method to Cambodian cultivars of indica type.

Materials and Methods

Female parents of 11 Cambodian cultivars of rice (Oryza sativa L.), Chheam An-tung, IR Kesar, Neang Minh, Phka Khgnei, Phka Mlis, Phka Rumdoul, Phka Rumchang, Rohat, Rumpe, Santepheap 3, Sen Pidao, were obtained from Cambodian Agricultural Research and Development Institute (CARDI). Koshihikari and W42 (a cultivar selected from the cross between Hinohikari and Koshihikari, Won et al., 1998) were used as male parents. Experiment was conducted at Utsunomiya University (Utsunomiya, Tochigi, Japan) in 2005 and 2006. Female plants were grown in pots under natural condition and were transferred to a dark room for exposing 8-hr photoperiod. During flowering time, the plants with about two third of panicles emerged out from the leaf sheath were selected as a female parent, and 5 panicles were used for each treatment. Spikelets that had already flowered before treatment and spikelets which didn’t open after treatment were removed.

1. Experiment of stigma tolerance to hot water

For experiment of stigma tolerance to hot water, panicles were submerged in hot water for 7 min at 43, 44, 45 and 46°C in 2005 and 44, 45 and 46°C in 2006. After the hot-water treatment, un-opened spikelets were removed, and 37 panicles each having more than 10 spikelets were artificially pollinated with un-treated healthy pollens of Koshihikari and W42. One week after pollination, the number of fertilized and non-fertilized spikelets was counted.

2. Experiment of pollen tolerance to hot water

For experiment of pollen tolerance to hot water, panicles were submerged in each treatment of hot water for 7 minutes at 40, 43 and 46°C in 2005 and at 40, 43, 44, 45 and 46°C in 2006 and self-pollinated. Then the panicles having more than 10 spikelets were chosen and grown in the natural condition, and covered with paper bags to avoid cross pollination. After seven days, the number of fertilized and unfertilized spikelets was counted. Koshihikari was

Fig. 1. Stigma tolerance treated, at 43~46°C in 2005, at 44~46°C in 2006 for 7 min were artificially pollinated by Koshihikari or W42 and the average of 3~7 panicles was shown. Bars indicate standard error.
included as a check cultivar.

**Results and Discussion**

The average percentage of fertilized spikelets of Cambodian cultivars per panicle tested for stigma tolerance treated at 43, 44, 45 and 46°C was 36.0, 23.6, 13.0 and 0.3%, respectively, in 2005 and at 44, 45 and 46°C, it was 21.8, 11.7 and 10.6%, respectively, in 2006 (Fig. 1).

The average percentage of self-fertilized spikelets of Cambodian cultivars per panicle tested for pollen tolerance treated at 40, 43 and 46°C was 17.8, 0.4 and 0.04%, respectively, in 2005 and at 40, 43, 44, 45 and 46°C, it was 14.4, 0.4, 0, 0.4 and 0.1%, respectively, in 2006 (Fig. 2). That of Koshihikari at 40, 43 and 46°C was 43, 0 and 3%, respectively, in 2005 and at 40, 43, 44, 45 and 46°C, it was 64, 0, 0.9, 1.4 and 0%, respectively, in 2006. The results of our experiments show that hot-water treatment at higher than 43°C for 7 minutes can be effective for inhibition of fertilization in Cambodian cultivars, although the treatment at 43–44°C did not inactivate the female organs in more than 20% of the spikelets.

In conclusion, hot water emasculation by 43°C for 7 minutes can be applied for artificial crossing of indica type Cambodian cultivars with japonica cultivars. A very few percentage (one or two grains) of fertilization over 43°C, which was also reported in Kondou (1939), might be practically negligible.

**References**

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* In Japanese

![Fig. 2. Pollen tolerance treated, at 40 - 46°C in 2005 and 2006 for 7 min were self-pollinated. Bars indicate standard error.](image)