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IDENTIFICATION OF WIDE COMPATIBILITY VARIETIES IN SOME TROPICAL JAPONICA RICE

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ABSTRACT

The F1 hybrid sterility in indica/japonica crosses is the major barrier in developing hybrid rice varieties between these two diverse germplasm. The sterility problem in japonica/indica hybrids can be overcome by using wide compatibility genes. The objective of this study was to identify wide compatibility varieties (WCVs) in some tropical japonica rice. Twenty five tropical japonica varieties as male parents were crossed with indica (IR64) and japonica (Akitakomachi) testers as female parents. The crosses were planted following a randomized complete block design with three replications. Varieties having average spikelet fertility of more than 70% with both the indica and japonica testers were rated as WCVs. Result from this study showed that six tropical japonica varieties were classified as WCVs, i.e., Cabacu, Grogol, Kencana Bali, Klemas, Lampung Lawer, and Napa. Hybrid sterility is caused by partial sterility of male and female gametes. The WCVs from the present study can be used in hybrid rice breeding program to solve hybrid sterility in indica/japonica hybrids.

[***Keywords***: *Oryza sativa*, tropical japonica, hybrid rice, hybrid sterility]

INTRODUCTION

The increase of rice production in Indonesia in the last decades was not significant; indeed trend in certain years was decreasing. The most potential approach to meet this challenge is through creating high yielding varieties.

The Green Revolution technology with semi-dwarf varieties has succeeded in meeting rice needs in the past three decades, but the increase has reached a plateau. Shifting the yield frontier in rice beyond the level of semi-dwarf varieties is considered an important strategy to meet this challenge. The International Rice Research Institute (IRRI) confirmed that hybrid rice offers potential option to increase varietal yields beyond the level of semi-dwarf rice varieties. Studies conducted at IRRI comparing the best hybrid rice with best inbred rice during 1986-1995 showed about 17% potential yield (Virmani 1999).

The extent of standard heterosis obtained in commercial rice hybrid is one of the critical factors that decide the economic viability of hybrid rice technology. Genetic diversity between parents, within a limit, is related to magnitude of heterosis in crosses derived from them (Virmani 1994). Two varietal types or groups, indica and japonica, have long been recognized in *Oryza sativa*. The japonica varieties are distributed over a wide range of latitude and divided into tropical and temperate subgroups. The tropical japonica is known as javanica (Oka 1991).

Hybrid rice varieties grown widely in China and those released in recent years for commercial cultivation in India, Vietnam, and Philippines are based on indica germplasm. These intervarietal hybrids showed a yield advantage of 15-20% over the best check varieties. In japonica rice hybrids cultivated commercially in China, standard heterosis is even lower (less than 10%) and, as a result, the area under such hybrids is limited. To raise the level of heterosis, Chinese and Japanese rice scientists proposed using indica/japonica crosses (Khush *et al*. 1998).

Hybrids derived from intergroup crosses are expected to have higher heterosis than intragroup crosses which means that potential yield of intergroup hybrids would be greater compared with intragroup hybrids. Exploitable heterosis has been maximum in indica/japonica crosses, followed by indica/tropical japonica and japonica/tropical japonica crosses. From the practical point of view, indica/tropical japonica hybrids would be an ideal choice for the tropics on account of their better adaptability (Virmani *et al*. 1997; Khush *et al*. 1998).

Commercial exploitation to enhance heterosis in wide crosses is hampered by intervarietal hybrid sterility. Genetic basis of this sterility is not understood yet. Several models have been proposed to explain the intervarietal sterility observed in the Asian cultivated rice. The two most influential models are the duplicated lethal model by Oka (1974) and the allelic interaction model by Ikehashi and Araki (1986). According to the duplicated lethal model, there are two loci, *S1* and *S2*, controlling gamete fertility, such that gametes carrying ++, +*s2* and *s1*+ are normal, and ones carrying *s1s2* are abortive. The allelic interaction model is simpler which involves three alleles at the *S-5* locus, i.e. S^i_5 (indica), S^j_5 (japonica) and S^n_5 (neutral). Zygotes formed of an S^n_5 allele with any of three alleles, i.e., $S^n_5S^i_5$, $S^n_5S^j_5$ and $S^n_5S^n_5$, have normal fertility, whereas zygotes of $S^i_5S^j_5$ are partially sterile. Thus the S^n_5 allele is compatible with both indica and japonica varieties, and the model, therefore, is also known as the wide compatibility model (Liu *et al*. 1996). Such varieties which posses a S^n_5 allele are called wide compatibility varieties (WCVs). Identification system for *S-5* is well constructed, with standard testers from indica and japonica types as well as such marker gene as *C* (chromogen for pigmentation) and *wx* (waxy endosperm) in linkage group I (Ikehashi *et al*. 1991). This study was conducted to identify WCVs in some tropical japonica rice varieties using tester from indica and japonica.

MATERIALS AND METHODS

Twenty five tropical japonica rice varieties were chosen for this study. To identify WCVs, these varieties as male parents were crossed with indica (IR64) and japonica (Akitakomachi) testers as female parents. The 50 F1 hybrids were planted in the field in the 2004 rice growing season at the Muara Experimental Station, Indonesian Institute for Rice Research, Bogor. The experiment was designed in a randomized complete block with three replications. Each plot contained 10 seedlings planted in a row. The space between plants in each row was 25 cm and plots were 25 cm apart.

The pollen and spikelet fertilities of the F1 hybrids of all the crosses were determined by a standard method (IRRI 1996). Pollen fertility was observed under a light microscope using iodine potassium iodide (I_2KI) staining method. Samples for pollen were collected from at least ten florets from individual plants at the sixth growth stage (heading). At least three microscopic fields were used to count fertile pollen grains (stained round) and sterile pollen grains (viz., unstained withered, unstained spherical, and partially stained round) (Fig. 1). Pollen fertility was calculated as ratio of fertile pollen to the total number of pollen grains (IRRI 1996). At maturity, five hybrid plants per plot were harvested and spikelet fertility for each plant was scored as the percentage of filled seeds in the total number of spikelets. Varieties having average spikelet fertility of more than 70% with both the indica and japonica testers were rated as WCVs (Malik and Khush 1996).

RESULTS AND DISCUSSION

Means of spikelet fertility and its standard deviation are presented in Table 1. The spikelet fertility of F1 hybrids with japonica tester (Akitakomachi) varied from 3.75% to 94.22%, while with indica tester (IR64) were 4.92% to 90.76%. Six varieties , i.e., Cabacu, Grogol, Kencana Bali, Klemas, Lampung Lawer, and Napa have average spikelet fertility more than 70% with both indica and japonica tester. These genotypes are presumed posses neutral allele S^n_5 in *S-5* locus, thus the crosses have a genetic constitution S^n_5/S^i_5 and S^n_5/S^j_5 with indica and japonica tester, respectively. These varieties can be rated as WCVs.

Several genotypes showed poor grain filling in the crosses with one of the tester, indica or japonica. Bulan

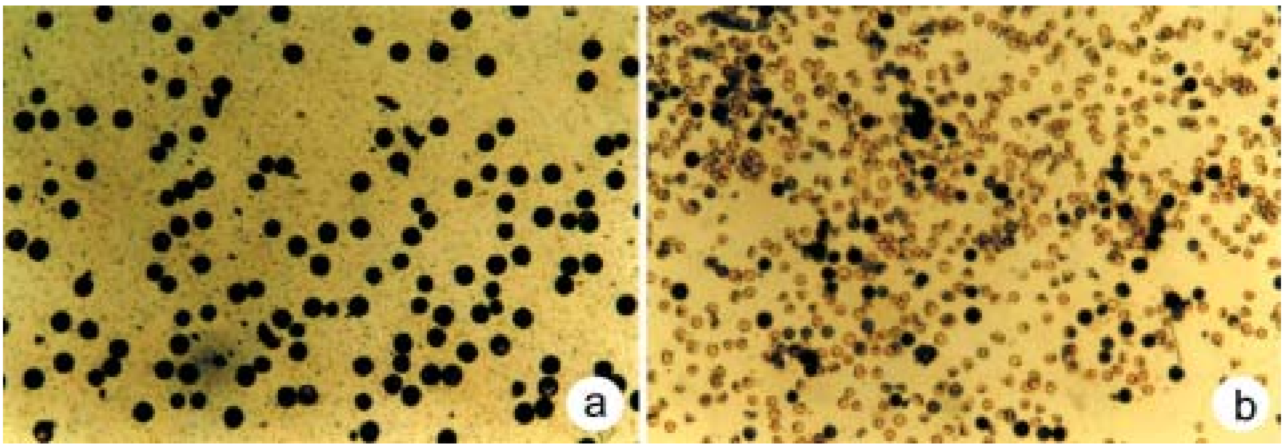

Fig.1. Pollen grains of rice stained by iodine potassium iodide (I_2KI) under light microscope (40x); a = normally fertile pollen grains (stained round pollen); b = partially sterile pollen grains (including unstained withered, unstained spherical and partially stained round pollen).

Table 1. Spikelet fertility (%) and its standard deviation of F1 hybrids of tropical japonica varieties with indica (IR64) and japonica (Akitakomachi) testers, Muara Experimental Station, Bogor, 2004.

Variety	Tester	
	Akitakomaci	IR64
Arias	93.57 ± 3.16	42.01 ± 5.66
Aselapan	90.37 ± 3.60	55.01 ± 1.36
Asemandi	91.83 ± 2.01	65.54 ± 4.25
Brentel	88.63 ± 3.57	56.97 ± 2.26
Bulan Sabit Putih	27.39 ± 8.88	85.67 ± 6.60
Cabacu	90.58 ± 5.97	83.24 ± 3.28
Dupa	90.80 ± 4.29	41.36 ± 1.75
Grogol	92.18 ± 3.81	88.05 ± 4.96
Hawara Bunar	88.65 ± 10.98	64.05 ± 2.75
Kencana Bali	81.55 ± 3.37	88.41 ± 6.20
Ketan Hitam	85.76 ± 5.62	68.75 ± 0.86
Ketan Merah	35.04 ± 5.25	4.92 ± 0.39
Ketan Merah Wangi	56.00 ± 2.66	76.49 ± 0.00
Ketupat	52.10 ± 3.47	87.10 ± 4.38
Kewal	3.75 ± 1.81	87.51 ± 5.36
Klemas	92.03 ± 4.85	75.14 ± 3.27
Lampung Kuning	94.22 ± 1.11	56.55 ± 1.28
Lampung Lawer	89.60 ± 5.12	78.42 ± 2.27
Lampung Putih	89.44 ± 6.76	59.65 ± 4.15
Leci	18.22 ± 5.96	82.91 ± 6.67
Mesir	89.41 ± 10.15	38.90 ± 0.28
Napa	91.16 ± 3.38	75.50 ± 1.33
Salumpikit	31.76 ± 6.18	82.11 ± 6.75
Sirendah Pulen	76.70 ± 22.29	53.55 ± 0.91
Way Rarem	19.53 ± 11.34	90.76 ± 1.13

Table 2. Pollen fertility (%) and its standard deviation of F1 hybrids of tropical japonica varieties with indica (IR64) and japonica (Akitakomachi) testers, Muara Experimental Station, Bogor, 2004.

Variety	Tester	
	Akitakomaci	IR64
Arias	90.58 ± 3.41	70.00 ± 1.25
Aselapan	87.08 ± 8.87	91.67 ± 2.35
Asemandi	79.17 ± 6.88	93.33 ± 2.60
Brentel	94.44 ± 4.49	95.42 ± 1.46
Bulan Sabit Putih	77.50 ± 9.01	97.58 ± 1.01
Cabacu	97.75 ± 1.52	87.89 ± 8.06
Dupa	95.33 ± 0.95	77.92 ± 3.61
Grogol	83.33 ± 4.39	96.08 ± 1.38
Hawara Bunar	98.25 ± 1.06	91.08 ± 3.50
Kencana Bali	78.89 ± 16.44	92.50 ± 5.45
Ketan Hitam	75.00 ± 21.21	97.00 ± 1.73
Ketan Merah	89.33 ± 8.22	44.17 ± 1.44
Ketan Merah Wangi	78.33 ± 5.77	88.56 ± 7.93
Ketupat	45.00 ± 18.03	93.50 ± 7.37
Kewal	19.17 ± 6.29	96.06 ± 3.87
Klemas	94.00 ± 2.63	79.58 ± 4.02
Lampung Kuning	96.39 ± 1.34	95.44 ± 1.50
Lampung Lawer	87.64 ± 9.52	93.50 ± 1.73
Lampung Putih	95.14 ± 3.05	92.08 ± 9.40
Leci	23.75 ± 6.96	88.67 ± 8.08
Mesir	93.92 ± 4.72	86.81 ± 4.26
Napa	89.75 ± 4.13	97.42 ± 0.63
Salumpikit	23.33 ± 16.50	91.42 ± 1.01
Sirendah Pulen	92.50 ± 7.07	92.33 ± 0.88
Way Rarem	31.25 ± 7.81	95.83 ± 3.97

Sabit Putih, Ketan Merah Wangi, Ketupat, Kewal, Leci, Salumpikit, and Way Rarem exhibited low (less than 70%) spikelet fertility in the crosses with japonica (Akitakomachi) tester. Referring to allelic interaction model by Ikehashi and Araki (1986), these low spikelet fertility genotypes were considered to posses S^i_5 allele, therefore the crosses with japonica have a genetic constitution S^i_5/S^j_5 which caused partial sterility of panicles. On the contrary, the genotypes Arias, Aselapan, Asemandi, Brentel, Dupa, Hawara Bunar, Ketan Hitam, Lampung Kuning, Lampung Putih, Mesir, and Sirendah Pulen were assumed carry S^j_5 allele because their F1 with indica (IR64) tester showed low spikelet fertility (Table 1).

An exception was demonstrated by Ketan Merah which exhibited low spikelet fertility in its crosses with japonica as well as with indica testers. Hybrid sterility in Ketan Merah which is not explained by the standard system for *S-5* alleles suggests that the tester for *S-5* are not applicable and the sterility may be caused at an additional locus. Ikehashi and Wan (1998) reported that a number of new hybrid sterility loci and five new loci (viz. *S-7, S-8, S-9, S-15, S-16* and *S-17*) have been identified. Allelic interaction at these loci can cause hybrid sterility in intervarietal hybrids, independently of each other, and neutral alleles to overcome this problem have been identified in different rice cultivars.

Pollen viability analysis was undertaken to determine whether hybrid sterility was caused by male or female gamete abortion. The pollen fertility of F1 with japonica tester (Akitakomachi) varied from 19.17% to 98.25% and with indica tester (IR64) were 44.17% to 97.58% (Table 2). The F1 hybrids, Akitakomachi/Bulan Sabit Putih, Akitakomachi/Ketan Merah, IR64/Arias, IR64/Aselapan, IR64/Brentel, IR64/ Dupa, IR64/Lampung Kuning, IR64/Lampung Putih, IR64/ Mesir, and IR64/Sirendah Pulen showed normal pollen fertility (more than 70%), but exhibited low spikelet fertility. The result indicated that the semi-sterility was caused only by female gamete abortion. This result similar to the report of Ikehashi and Araki (1986) in which genetic mechanism of hybrid sterility in Asian rice cultivars involved egg-killer and induced abortion of megaspore. In the heterozygote ($S^i_5S^j_5$), an S^i_5 allele from the indica parent induces the abortion of megaspores carrying the other allele (S^j_5 from japonica), suggesting that the S^i_5 allele may act as an egg-killer against S^j_5.

In another F1 hybrids (Akitakomachi/Ketupat, Akitakomachi/Kewal, Akitakomachi/Leci, Akitakomachi/Salumpikit, Akitakomachi/Way Rarem, and IR64/Ketan Merah), both pollen fertility and spikelet fertility were low (<70%). Thus, the hybrid sterility in these crosses is not only caused by female gamete abortion, but also by male gamete abortion. Sano (1993) reported instead of an egg-killer, there are different types of sterility gene(s) determining hybrid sterility. Zichao *et al*. (1996) also showed the partial sterility in the indica-japonica crosses appeared not only in male gamete but also in female gamete possibly with similar degree. Further attempts will be necessary to incorporate S^n_5 allele into parental lines of hybrid rice to develop commercial hybrid rice derived from indica and japonica varieties.

The present study indicates that WCVs should be used to solve the problem of hybrid sterility in indica-japonica crosses. Incorporation of S^n_5 allele into cytoplasmic male sterile (CMS) and restorer lines, the parental lines of hybrid rice, is important to achieve an efficient exploitation of the strong heterosis of hybrids derived from indica and japonica varieties.

CONCLUSION

Based on allelic interaction model, six wide compatibility varieties (WCVs) have been identified, i.e. Cabacu, Grogol, Kencana Bali, Klemas, Lampung Lawer, and Napa. These varieties can be used in hybrid rice breeding program to arrive an efficient exploitation of the strong heterosis between indica and japonica varieties. In the present study, semi-sterility in the inter-varietal crosses is caused by partial sterility of male and female gametes.

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