Sex Plant Reprod (2004) 17: 55–62
DOI 10.1007/s00497-004-0214-z

ORIGINAL ARTICLE

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Male and female gamete abortions, and reduced affinity between the uniting gametes as the causes for sterility in an indica/japonica hybrid in rice

Received: 27 January 2004 / Accepted: 15 March 2004 / Published online: 6 May 2004

Abstract Hybrid sterility frequently occurs in crosses between indica and japonica subspecies of Asian cultivated rice. In this study, we investigated the cytological processes involved in formation and development of male and female gametes as well as their interactions in fertilization, using an indica/japonica hybrid in comparison with an indica/indica hybrid. It was found that more than 50% of the microspores generated in the indica/japonica hybrid could not develop into functional pollen. The abortion rate of microspores in the indica/japonica hybrid was much higher than that in the indica/indica hybrid. Abortive embryo sacs made up roughly 70% of the embryo sacs examined in the indica/japonica hybrid, which was also much higher than that detected in the indica/indica hybrid. Moreover, the amount of pollen adherence on stigmas of the indica variety upon hand-pollination with pollen from the japonica variety was much lower than the indica/indica pollination, and the number of pollen adhered on the stigmas by natural self-pollination was much greater in the indica/indica hybrid than in the indica/japonica hybrid. The indica/japonica hybrid also encountered difficulties in pollen tube growth after pollination, and the fertilization rate of the indica/japonica hybrid was much lower than that of the indica/indica hybrid. These results clearly illustrate the complexity of the mechanisms underlying inter-subspecific hybrid sterility in rice involving both pre- and post-zygotic reproductive isolation mechanisms.

Keywords *Oryza sativa* · Indica/japonica hybrid sterility · Microspore · Megaspore · Fertilization

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Introduction

Hybrid sterility frequently occurs in crosses between indica and japonica subspecies of Asian cultivated rice (*Oryza sativa* L.) (Kato et al. 1928; Oka 1988). It is known that the majority of the indica/japonica hybrids are partly sterile, with the spikelet fertility mostly in the range of 30–60% (Liu et al. 1996). It has also been observed that strong heterosis exists in many indica/japonica hybrids in both vegetative and reproductive growth, which has attracted the attentions of rice breeders for several decades (Yang et al. 1962; Chu et al. 1964; Yuan 1987). Consequently, there has been considerable interest in understanding the causal mechanisms that lead to the hybrid sterility observed in indica/japonica crosses.

The genetic basis of the inter-subspecific hybrid sterility has been extensively investigated in recent decades. Based on the analysis of near isogenic lines, Oka (1974) proposed a "duplicated lethal" model that involved s-alleles at two genetically duplicated loci to explain the genetic basis of the hybrid sterility, whereas Ikehashi and Araki (1986), based on the finding and analysis of wide compatibility varieties that are able to produce normal fertility hybrids with both indica and japonica varieties, proposed "allelic interaction" as the genetic explanation for indica/japonica hybrid sterility. Recent studies, making use of molecular marker technology and high density molecular marker linkage maps, have not only confirmed the loci that cause hybrid sterility as identified by both Oka (1974) and Ikehashi and Araki (1986), but also precisely determined the locations of these loci in the rice genome (K.D. Liu et al. 1997; Zhuang et al. 1999, 2002). Moreover, a series of additional loci were also identified as causing gamete abortions or hybrid sterility in indica/japonica crosses (Ikehashi and Wan 1996; Wang et al. 1998).

Cytological mechanisms of inter-subspecific sterility have also been investigated, and have produced variable results depending on the interests of the investigators, such as male gamete abortions (Wang et al. 1991; He et al. 1994; Teng et al. 1996; Zhu et al. 1996), female gamete

abortions (Yokoo 1984; Li 1988; Ling et al. 1991; Li and Ouyang 1992; Wang et al. 1992; Y.S. Liu et al. 1993, 1997; Zhu et al. 1996), and reduced dehiscence of the anthers (Maeka et al. 1991; Liu et al. 1993). It has also been reported that environmental conditions, especially low temperature, can greatly reduce the fertility of the inter-subspecific hybrids, while the same temperature may not have significantly adverse effects on the fertility of intra-subspecific hybrids (Qi et al. 1993; Li et al. 19961997; Lu et al. 2002). Thus, despite the large number of studies conducted thus far, the mechanism of the indica-japonica hybrid sterility is still an issue of debate.

In the study reported in this paper, we investigated the cytological processes underlying the formation and development of both microspores and megaspores, and fertilization, in an indica/japonica hybrid in comparison with an indica/indica hybrid. The objective was to identify, at the cytological level, the causal mechanism for the partial sterility in the indica/japonica hybrids.

Materials and methods

Genetic materials

The genetic materials used in this study were two rice cultivars (Nanjing 11 and IR36) of *O. sativa* ssp. *indica*, and one cultivar (Akihikari) of *O. sativa* ssp. *japonica*. These three varieties are regarded as typical indica or japonica, and were recommended and used as testers for compatibility analyses (Liu et al. 1996). Nanjing 11 was crossed with Akihikari to produce an inter-subspecific hybrid, and with IR36 to produce an intra-subspecific hybrid. The parents and the hybrids were grown in 2001 and 2002 in the Experimental Farm of Huazhong Agricultural University, Wuhan, China.

Observing abortion during male and female gamete formation

The processes of stamen and pistil development were examined using a microscope. For preparing the microscopic slides, florets were collected from top, middle and bottom parts of each panicle, during the period from pistil and stamen differentiation of the young panicle development to 1 or 2 days before flowering. The harvested florets were fixed in a solution containing an 18:1:1 mixture of 70% ethanol, formalin and acetic acid. Anthers and ovaries were collected from the fixed floret tissue, stained with Ehrlich's haematoxylin and dehydrated with ethanol. After embedding in Paraffin, the tissue was sliced into 8-μm-thick slices, and microscope slides were prepared following the procedures described by Sun and Qian (1987) and Wang et al. (1992). The slide was viewed and photographed using a photomicroscope (Olympus BH-2).

Observation of affinity between pollen and stigma

The affinity between pollen and stigma was examined by observing the behavior of the pollen grains on the stigma after pollination. To prepare the tissues, the maternal plants were emasculated by incubating the panicle in water at 45°C for 5 min, followed by anthers removal using a pair of forceps. The emasculated panicles were bagged and pollinated with pollen from the respective paternal parents. From each hybrid, 20–30 florets were collected 30 min after pollination, fixed in 3% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.1), and stored at 0–4°C for 6–7 days. Before dehydrating, panicles were washed using an ethanol series of 30%, 50%, 70%, 80%, 90% once each, followed by 100% twice for 15–30 min each, and then isoamyl acetate once for 15–30 min. The panicles were critical-point dried with CO_2 and covered with gold using a sputter coater (EikoIB5, Hitachi, Japan). Adherence, germination and growth of the pollen on the stigma was examined by observing fixed tissues of pollinated stigmas using a scanning electron microscope (S-450, Hitachi, Japan) (Lan and Xu 1996).

Determining pollen fertility

Ten panicles per hybrid were sampled at 1 or 2 days before flowering to determine pollen fertility of the hybrids. Six florets per panicle were taken from the upper, middle and lower portions of the panicle. One anther per floret was collected, and the six anthers from the same panicle were mixed and spread on a slide. Four views per slide were observed with a microscope, resulting in 40 views per hybrid. Two methods were employed to examine pollen fertility. In the first, the pollen grains on the slide were stained with 1% IK-I_2 and the darkness of the staining was used as an indicator of pollen fertility; in the second, a drop of fluorescein diacetate (FDA) solution was placed on a slide to which pollen had been dusted. After staining for 5 min, the slide was observed using an epifluorescence microscope, with the assumption that intense fluorescence is correlated with the pollen activity (Heslop-Harrison 1975).

Determining the fertilization status of embryo sacs

The florets were sampled at 1 day after flowering (or pollination), and incubated overnight in warm water (about 40°C). After soaking

Table 1 Comparison of abnormality between the Nanjing 11/Akihikari and Nanjing 11/IR36 hybrids observed during consecutive stages of microspore formation

Cross		Numbers of anthers examined in different stages of microspore formation					
		Sporogenous cell	Microsporocyte	Dyad	Tetrad	Microspore	Mature pollen
Nanjing 11/Akihikari	Total	336	285	189	174	148	115
	Normal	335	276	189	174	118	58
	Abnormal	1	9	0	0	30	17
	% Abnormal	0.30	3.16	0	0	20.27	49.56
Nanjing 11/IR36	Total	358	327	265	247	222	207
	Normal	358	327	265	247	217	178
	Abnormal	0	0	0	0	5	29
	% Abnormal	0	0	0	0	2.25	14.01

in 35% H_2SO_4 for 30 min followed by rinsing in water, the florets were transferred to an IK-I_2 solution and stained for another 30 min. The stained florets were rinsed with 70% ethanol, kept in 95% or 100% ethanol for at least 30 min, and then treated with xylene for crystallization before they were examined.

Results

Abnormality in male gamete formation of the inter-subspecific cross

For ease of description, we divided the process of microsporogenesis into four continuous stages: sporogenesis, formation of microsporocytes (pollen mother cells), meiosis, formation of microspores and pollen maturation. During sporogenesis, the anther is differentiated into four anther sacs separated by the connective tissue, and usually four sporogenous cells are formed in each of the anther sacs. The sporogenous tissues were normal in the indica/indica (Nanjing 11/IR36) hybrid, but abnormality occurred occasionally in the Nanjing 11/Akihikari cross in which the chromatin of sporogenous cells became darkly stained (Fig. 1a).

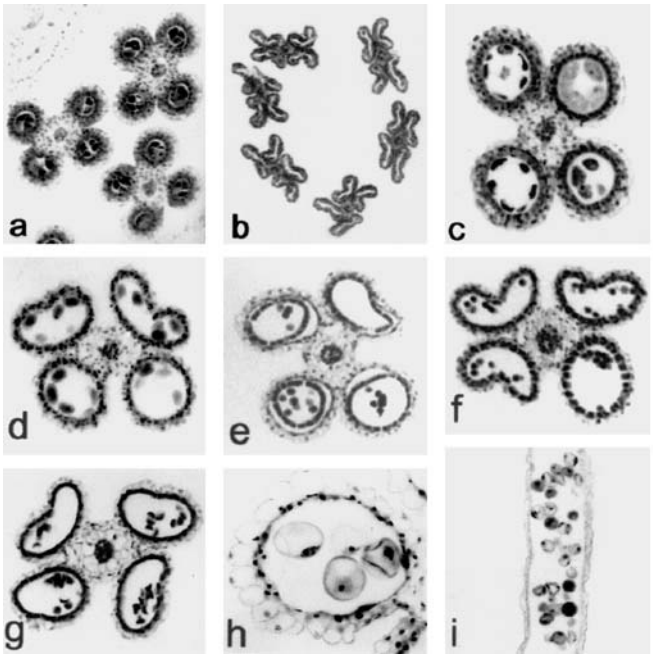

Fig. 1a–i Various abnormalities observed during the process of microsporogenesis and development in the *Oryza sativa* L. cv. Nanjing 11/Akihikari hybrid observed using Paraffin-embedded slides. **a** Sporogenous cells became darkly stained and chromatin-rich during microsporogenesis. **b–d** Abnormalities observed during microspore mother cell formation: **b** deformed anthers with degenerated microspore mother cells stain darkly; **c** anthers appear to be normal but many of the microspore mother cells were degenerated and darkly stained; **d** one or more anther chambers were deformed, while most of the microspore mother cells were normal. **e–g** Abnormalities observed during early microspore formation: e anther walls were badly deformed and tapetum cells dissociated from the middle layer, while microspores were mostly normal; **f** anther walls were badly deformed and microspores were darkly stained; **g** tapetal layers were still not disintegrated while the microspores were misshaped. **h** Delayed disintegration of the tapetum layer resulted in sterile microspores at the uni-nucleate stage. **i** During pollen maturation, most of the pollen grains were not round and plump

Normal microsporocytes are characterized by their large volumes and large nuclei with no visible tonoplasts, which is the case for the indica/indica hybrid. However, occasional abnormality was observed in the inter-subspecific hybrid, in which the chromatin in microsporocytes was darkly stained, and in some cases accompanied by deformation of the anthers (Fig. 1b–d). These abnormalities made up 3.16% of the 285 anthers examined in the Nanjing 11/Akihikari hybrid, while not a single abnormal case of this kind was observed in a total of 327 anthers examined in the Nanjing 11/IR36 hybrid.

No abnormality was detected during meiosis in either of the crosses until the stage of tetrad formation, after which two kinds of abnormalities were observed in the Nanjing 11/Akihikari hybrid: (1) during the separation of the tetrads into individual microspores, the anthers occasionally became deformed in the Nanjing 11/Akihikari cross (Fig. 1e–g), presumably due either to the premature (Fig. 1e) or delayed separation of tapetum cells from the middle layers (Fig. 1g), eventually leading to abortion of the microspores. (2) At the stage of a single nucleus to two nuclei of microspore development, a large proportion of microspores in the Nanjing 11/Akihikari hybrid became abortive as indicated by their irregular shape (Fig. 1h). This is again largely due to incorrect timing of tapetum disintegration (too early or too late), which interrupted the nutrient supply to the developing microspores. Further abortion of microspores also took place during pollen maturation (Fig. 1i). Consequently, the frequency of pollen abortions was much higher in the Nanjing 11/Akihikari cross than in the Nanjing 11/IR36 hybrid (Table 1).

Abnormality in female gamete formation of the inter-subspecific cross

No visible abnormality in either of the hybrids was detected during the process from archespore to the formation of megaspores. Two types of abnormalities became visible at the stage of megaspores developing into embryo sacs. In one type, the megaspores failed to differentiate, leading to the failure of embryo sac formation. In this case, only a clump of dark-stained cells was observed in the ovule (Fig. 2a). As the second type of abnormality, the developmental process of megaspores into embryo sacs was not completed, resulting in degenerated cells lying on the chalazal side (Fig. 2b). The occurrence of these abnormalities was due largely to the inability of the developing megaspore cells to absorb the degenerating nucellus cells, and consequent inability to grow to form the embryo sacs. The frequencies of the two types of abnormalities in the inter-subspecific hybrid Nanjing 11/Akihikari (12.68%) were much higher than that in the Nanjing 11/IR36 hybrid (0.85%).

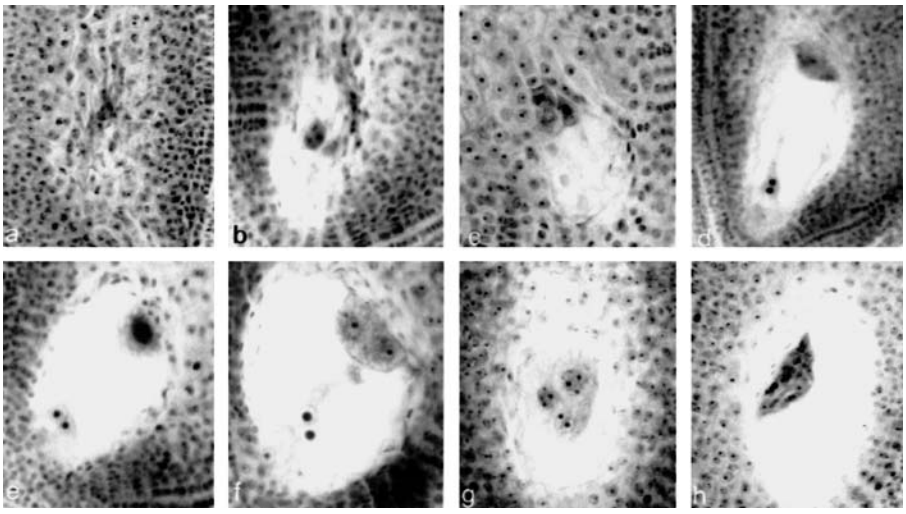

Fig. 2a–g Various abnormalities observed during the process of megasporogenesis and development in the Nanjing 11/Akihikari hybrid in Paraffin-embedded slides. **a** Embryo sacs did not differentiate and darkly stained degenerated embryo sac and nucellus cells were visible in the ovule. **b** Embryo sac differentiation was not completed, and the structure of the embryo sac was visible on the micropylar side, with degenerated cells on the chalazal side. **c–f** Abnormalities observed in nuclear division during megagametogenesis: **c** three-celled embryo sac, **d** five-celled embryo sac, **e** six-celled embryo sac, **f** seven-celled embryo sac. Normal antipodals of the Nanjing 11/IR36 hybrid (**g**) abnormally arranged antipodals of the Nanjing 11/Akihikari hybrid

As the development of the embryo sac continues, the megaspore normally divides three times to form an embryo sac of eight nuclei. The three nuclei near the micropyle form one egg cell and two synergids, and the three nuclei near the chalaza develop into antipodals, while one nucleus from each side moves to the center to form the central cell with two polar nuclei. While abnormality was rarely observed in the process of megaspore divisions in the formation of the eight-nucleus embryo sac in the Nanjing 11/IR36 hybrid, a large proportion of abortive embryo sacs occurred in the Nanjing 11/Akihikari hybrid. In most cases, Nanjing 11/Akihikari hybrid was unable to complete the second or third division. Consequently, the normal eight-nucleus embryo sacs were observed in only 22 (30.99%) of the 71 cases in which embryo sacs were observable on the slides. Embryo sacs with 2–7 nuclei made up of 56.34% of the 71 observable cases (Fig. 2c–f; Table 2). Clearly, failures in the divisions were largely responsible for embryo sac abortion in the inter-subspecific hybrid.

We also observed abnormality in the antipodals. Normally, antipodals in rice are composed of a group of cells resulting from continuous divisions of the antipodals (Hu 1982). In the Nanjing 11/IR36 hybrid, there were three small clusters of antipodals, each of which was neatly arranged with a prominent nucleus, with the cells plump and relatively uniform in size (Fig. 2g). In the Nanjing 11/Akihikari hybrid, the antipodals were wrinkled, and their arrangement unordered (Fig.2 h). This may also partly account for the embryo sac abortion observed in this cross.

Pollen fertility

Stainability by KI-I_2 reflects the amount of starch accumulated in the pollen, and is highly correlated with pollen fertility. In general, darkly stained pollen is regarded as highly fertile (Fig. 3a), while those lightly stained or unstained are sterile (Fig. 3b, c). Approximately 25–30% of the pollen of the Nanjing 11/Akihikari hybrid was darkly stained, while the proportion of darkly stained pollen was 78–94% in the Nanjing 11/IR36 hybrid (Table 3).

Stainability with FDA mediated by the activity of esterase in the pollen was also assessed. Again, the two hybrids differed greatly in their FDA stainability (Table 3). Class I and II FDA staining accounted for over 80% of the pollen in the Nanjing 11/IR36 hybrid (Fig. 3d), while these two classes of staining made up only about 45% of the pollen in the Nanjing 11/Akihikari hybrid (Fig. 3e).

Table 2 Comparison of abnormality between the Nanjing 11/Akihikari and Nanjing 11/IR36 hybrids observed during consecutive stages of embryo sac formation and development

Cross		No differentiation	2–4 nucleus	5–7 nucleus	Sub-total	8-nucleus (normal)	Total
Nanjing 11/Akuhikari	Number observed	9	21	19	49	22	71
	%	12.68	29.58	26.76	69.01	30.99	
Nanjing 11/IR36	Number observed	1	7	11	19	99	118
	%	0.85	5.95	9.32	16.10	83.90	

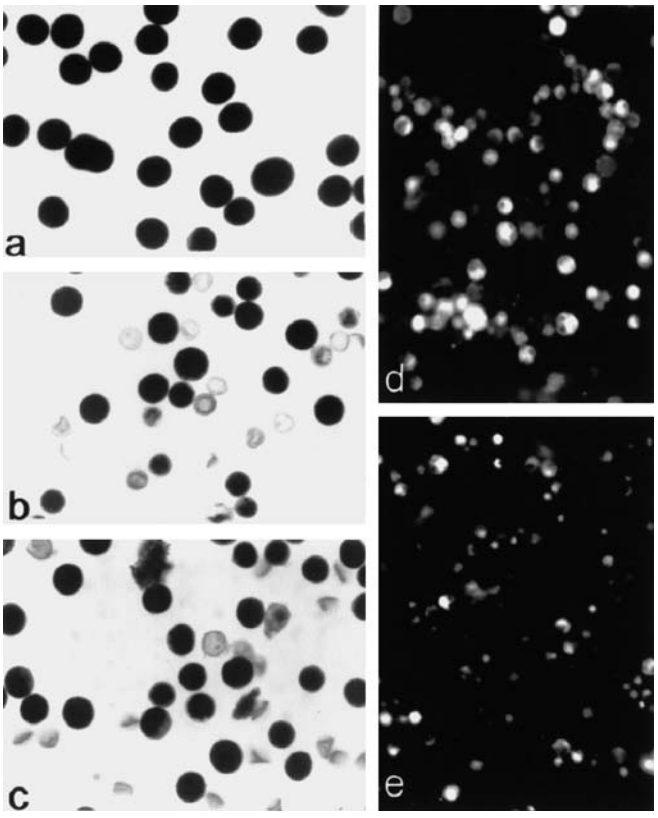

Fig. 3a–e Pollen fertility. **a** Mature pollen of the Nanjing 11/IR36 hybrid stained with IK-I_2 showing that all pollen are fertile. **b**, **c** Mature pollen of the Nanjing 11/Akihikari hybrid stained with IK-1_2 showing that large portions of the pollen are sterile. **d** Mature pollen of the Nanjing 11/IR36 hybrid stained with fluorescein diacetate (FDA) showing that most of the pollen had high activity. **e** Mature pollen of the Nanjing 11/Akihikari hybrid stained with FDA showing that very few pollen had high activity

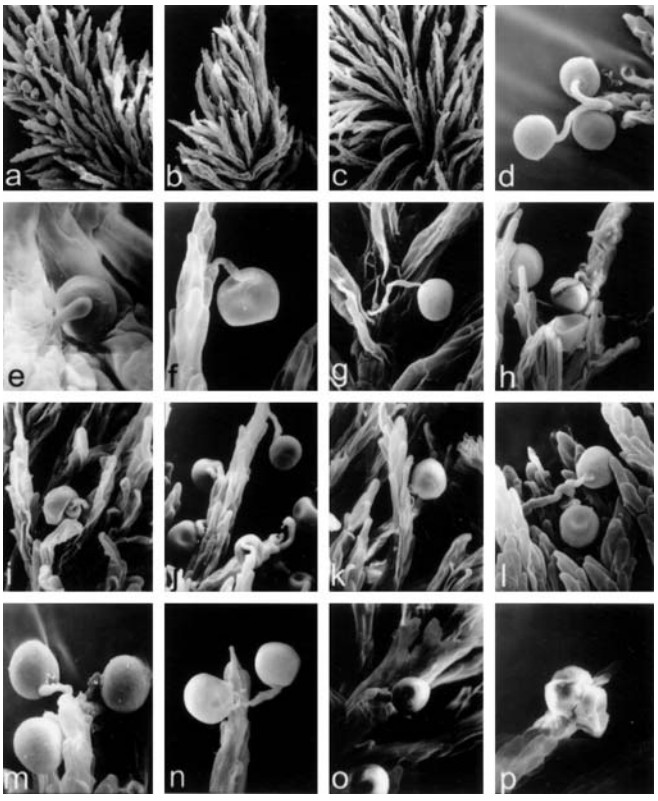

Fig. 4a–p Adherence, germination, and growth of pollen tubes on stigmas observed using scanning electron microscopy. **a** Adherence of numerous pollen grains on stigmas of the Nanjing 11/IR36 hybrid. **b**, **c** Few or no pollen grains adhered on the stigmas of the Nanjing 11/Akihikari hybrid. **d** Vigorous pollen grains and pollen tubes grown on stigmas of the Nanjing 11/IR36 hybrid. **e–p** Various abnormalities in germination and growth of the pollen tube on stigmas of the Nanjing 11/Akihikari hybrid

Thus, the Nanjing 11/IR36 hybrid clearly has much higher pollen fertility as assayed by both KI-I_2 and FDA staining. These levels of pollen fertility were also consistent with the spikelet fertility of these two hybrids (Table 3).

Adherence and growth of pollen on the stigma

The amount of pollen adherence on stigmas of the Nanjing 11/Akihikari cross was much less than with Nanjing 11/IR36 in both the F_0 (crossing between the two parents) and F_1 (pollination of the hybrid using its own pollen) crosses, with artificial pollination. Large numbers of pollen adhered to the stigma of the Nanjing 11/IR36 cross (Fig. 4a), with an average of 16.7 pollen per stigma in the F_0 cross and 21.4 in the F_1 hybrid, while in the Nanjing 11/Akihikari cross, the average pollen adhered was 2.25 per stigma in F_0, and 1.75 in F_1, with no or very few pollen observed on many of the stigmas (Fig. 4b, c). This suggested that the affinity between pollen and stigmas was much lower in the Nanjing 11/Akihikari cross than in the Nanjing 11/IR36 cross, and the affinity was even lower in the F_1 than F_0 of the Nanjing 11/Akihikari cross.

The stigmas of the F_1 of the two hybrids were examined 30 min after pollination. The majority of the pollen could

Table 3 Pollen fertility of the two hybrids. FDA Fluorescein diacetate

Cross		KI-I_2stainability (%)			FDA stainability (%)				Seed setting (%)
		Dark stain	Light stain	Unstained	I	II	III	IV	
Nanjing 11/Akihikari	2001	25.95	22.94	51.11	27.35	17.39	15.05	40.21	27.05
	2002	30.69	15.74	53.57					26.68
Nanjing 11/IR36	2001	77.87	7.09	15.04	47.52	35.88	5.13	11.47	90.22
	2002	94.11	1.62	4.27					64.08

Fig. 5 Fertilized (*right*) and unfertilized (*left*) ovaries with IK-I_2 staining after crystallization treatment by HCl

germinate on the stigmas, and the pollen tubes of most of the germinated pollen could elongate. However, the degree of pollen tube elongation differed greatly between the two hybrids. In the Nanjing 11/IR36 hybrid, the pollen tubes were round, straight and smooth looking. At the contact points of pollen tubes on the stigmas, the cells of the stigma surface were altered and became wrinkled (Fig. 4d), indicating rapid interactions between substances released from the pollen tubes in the stigma. Various abnormalities of pollen tube growth were observed in the Nanjing 11/Akihikari hybrid (Fig. 4e–n), presumably due to some protecting mechanism of the stigmas against pollen resulting in non-affinity reactions.

Fertilization rates

Blue staining of the ovary, which could be observed after the crystallization treatment of the glumes, indicated a fertilized ovary (Fig. 5). The frequencies of fertilized and unfertilized ovaries in the 2 years are listed in Table 4. Although there were large fluctuations in the rates of fertilization of the ovaries over the 2 years due to the weather conditions, the two hybrids nonetheless differed greatly in their rates of fertilization. Again, the proportion of fertilization of ovaries in the Nanjing 11/IR36 hybrid was much higher than in the Nanjing 11/Akihikari hybrid.

Relationships between pollen adherence, fertilization and seed-setting

We examined the relationships between pollen adherence, fertilization and seed-setting rates (Table 5). The amount of pollen adherence on the stigma was highly correlated with both the rates of fertilization (r=0.94) and seed-setting (0.89). Seed-setting rate was also highly correlated with fertilization rate (0.99). The latter correlation was significant at the 0.01 probability level, while the other two correlations were not statistically significant due to the small degrees of freedom. This clearly indicated that fertilization and seed setting were highly dependent on adherence of pollen on the stigma.

Discussion

We have examined the processes of male and female gamete formation and development in two crosses, Nanjing 11/IR36 and Nanjing 11/Akihikari, presumably representing typical inter- and intra-subspecific hybrids, respectively. The results have revealed a number of interesting features related to the inter-subspecific hybrid sterility that is frequently observed in indica/japonica hybrids.

During the process of microspore formation and development, the inter-subspecific hybrid was mostly normal up to the stage of meiosis and tetrad formation, with abortion occurring largely at the one-nucleus stage, primarily due to incorrect timing of the degeneration of the tapetum cells. Such observations are consistent with the reports of Laser and Lersten (1972), Ren and Zhou (1990)

Table 4 Rates of fertilized ovaries in the two hybrids

Cross	Year	Number of florets assayed	Fertilized		Unfertilized	
			Number	%	Number	%
Nanjing 11/Akihikari	2001	3,090	1,017	32.91	2,073	67.09
	2002	2,951	770	26.09	2,181	73.91
Nanjing 11/IR36	2001	3,014	2,636	87.46	378	12.54
	2002	3,031	1,882	62.09	1,149	37.91

Table 5 Adherence of pollen on stigmas, fertilization and seed-setting rates in various crosses with artificial emasculation and pollination

	Cross	Number of pollen per stigma	Fertilized ovaries (%)	Seed-setting rate (%)
F_0	Nanjing 11/Akihikari	2.25±1.38**	47.65±20.23**	24.41±15.80*
	Nanjing 11/IR36	16.70±4.11	93.88±37.91	34.83±15.26
F_1	(Nanjing 11/Akihikari)/(Nanjing 11/Akihikari)	1.75±1.23**	32.91±17.08**	17.92±11.30**
	(Nanjing 11/IR36)/(Nanjing 11/IR36)	21.40±9.50	87.46±32.62	32.12±14.27

*Difference between the intra- and inter-subspecific hybrids not significant at the 0.05 probability level
**Indica/japonica hybrid lower than indica/indica hybrid at the 0.01 probability level

and Wang et al. (1991). Consequently, more than 50% of the microspores generated in the inter-subspecific cross cannot develop into functional pollen. This rate is 3–4 times higher than the abnormality observed in the intra-subspecific cross. Pollen activity tests further confirmed that the proportion of fertile pollen produced by the intra-subspecific hybrid was approximately three times higher than that produced by the inter-subspecific hybrid.

Similarly, during the formation and development of female gametes, both intra- and inter-subspecific crosses appeared to be normal in meiosis, and also up to the point of formation of functional megaspores. Our observations suggest that the abnormality becomes apparent at or after the first cell-division during the process of embryo sac formation and development. This is consistent with the findings of Oka (1957), who speculated that abortion occurred when the megaspore started the first mitotic division, and of Zhu et al. (1996), who suggested that abortion occurred at the two-nucleus stage. Y.S. Liu et al. (1997) examined F_1 crosses of 15 indica-japonica hybrids and found that abortion could occur even before the first division of the megaspore, and also during the first and second divisions. However, our results certainly differ from those of Ouyang and Li (1992), who asserted that embryo sac abortion occurred as a continuous process that could be detected at all stages. The abortive embryo sacs made up roughly 70% of the embryo sacs examined in the inter-subspecific cross, a rate that is also about four times higher than the abnormality detected in the intra-subspecific cross.

Xu (1995) observed that the amount of pollen grains adhered to the stigmas of a japonica variety was much smaller upon hand pollination with pollen from an indica variety or vice versa, than that of pollinating indica on indica, or japonica on japonica, or a wide compatibility variety on either indica or japonica. The present study also revealed that the amount of pollen adherence on the stigmas of the indica variety pollinated using pollen from the japonica variety was much smaller than with the indica/indica pollination. In addition, we also observed large differences in the amounts of pollen adherence on the stigmas between the intra- and inter-subspecific hybrids upon hand pollination. Further differences were also detected during the fertilization process. The inter-subspecific hybrid also encountered difficulties in pollen tube growth after pollination, similar to the observation of Xu (1995) who also observed difficulty in growth of indica pollen on japonica stigmas, and vice versa. These difficulties in pollen adherence and pollen tube growth resulted in large differences in the rates of fertilization between intra- and inter-subspecific hybrids. This clearly suggests a different level of affinity between pollen and stigmas in intra- and inter-subspecific pollinations.

These results clearly demonstrated the complexity of the mechanisms underlying inter-subspecific hybrid sterility in rice, which can be described as a "syndrome" involving a variety of phenomena including both pre-zygotic reproductive isolation due to the reduced affinity between the pollen and stigmas of the two subspecies, and post-zygotic reproductive isolation or hybrid dysgenesis featured by both male and female gamete abortions, as well as reduced affinity between pollen and stigmas of the F_1. The relative importance of each of these components in inter-subspecific sterility, and their underlying causes, remain to be determined in future studies.

Acknowledgement This research was supported by a grant from the National Special Key Project on Rice Functional Genomics.

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