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Mapping QTLs for defective female gametophyte development in an inter-subspecific cross in *Oryza sativa* L.

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Abstract The embryo-sac is an essential structure for angiosperm reproduction. The cytological and genetic characterization of embryo-sac sterility was examined in a cross between *Oryza sativa* ssp. *indica* cv. ZYQ8 and ssp. *japonica* cultivar, JX17. The arrest of embryo-sac development was manifested following meiosis in the F_1 hybrid. When the megaspore carried the lethal genotype, the nucleus either failed to divide or divided only once, and the immature embryo-sac degenerated. Abortion of the embryo-sac in the *indica-japonica* hybrid background was not observed in their original parents, and an effect of cytoplasmic gene(s) on embryo-sac sterility in the reciprocal F_1 hybrids was not detected. Using a rice molecular linkage map based on a doubled haploid (DH) population from the cross of ZYQ8 /JX17, we mapped quantitative trait loci (QTLs) for the defective development of the female gametophyte in backcross progenies from the DH lines. The result demonstrated that a polygenic system is involved in both megagametogenesis and postzygotic isolation in inter-subspecific hybrid rice.

Keywords Hybrid sterility · Megagametogenesis · Embryo-sac development · Postzygotic isolation · *Oryza sativa* L.

Introduction

Strong hybrid vigor has been frequently observed in the F_1s between the two subspecies of the Asian cultivated rice (*Oryza sativa* L.), ssp. *indica* and ssp. *japonica*. However, a major difficulty encountered in developing such inter-subspecific hybrid rice is the postzygotic reproductive barriers that are manifested as hybrid sterility and significantly reduced fertility in the F_1 and successive generations and backcrosses to parental characters (Kato et al. 1928; Kitamura 1962; Oka 1974, 1988; Ikehashi and Araki 1986; Li et al. 1997; Li and Yuan 2000). It is the differentiation between the *indica* and *japonica* rice groups that causes the postzygotic isolation between the subspecies. Such *indica-japonica* differentiation has been detected in various rice samples using several classes of markers, including biochemical and morphological traits (Oka 1988), isozymes (Glaszmann 1987), DNA restriction fragment length polymorphism (RFLP) (Zhang et al. 1992), simple sequence repeats (SSRs) (Yang et al. 1994; Blair et al. 1999), randomly amplified polymorphic DNA (RAPD) (Mackill 1995), amplicon length polymorphism (ALP) (Xu et al. 1998) and some unique DNA sequences (Oba et al. 1996). Hybrid sterility is commonly observed in many crosses between *indica* and *japonica* varieties (Oka 1988). Although a number of loci that are involved in hybrid sterility in different *indica-japonica* crosses have been located on the rice linkage map (Ikehashi and Araki 1986; Oka 1988; Yanagihara et al. 1995; Wan et al. 1996; Liu et al. 1997b; Zhang et al. 1997; Li et al. 1997), such studies have been largely based on data collections obtained from the pollen fertility and/or seed set rate. There is no study focusing on the phenotyping of embryo-sac morphology due to the difficult accessibility of the embryo-sac, which is buried deep within the sporophytic tissues of the ovule.

The female gametophyte (embryo-sac or megagametophyte) plays a pivotal role in sexual reproduction of angiosperms. It is the structure that produces the egg cell and central cell which, following fertilization, give rise to the seed embryo and endosperm, respectively (Maheshwari 1950). In addition, the female gametophyte mediates a host of reproductive processes including pollen-tube guidance, fertilization and the induction of seed development (Reiser and Fischer 1993; Ohad et al. 1996;

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Ray et al. 1997). Female gametophytic development comprises three synchronous rounds of free-nuclear mitosis followed by cellularization that results in a seven-celled embryo-sac (Webb and Gunning 1990; Reiser and Fischer 1993; Grossniklaus and Schneitz 1998). Several major events occur during megagametogenesis, including syncitial nuclear divisions, cellularization, nuclear migration and fusion and cell death. Deficiency analysis and transmission studies suggest a large number of genes are required during the haploid gametophytic phase (Patterson 1978; Buchner and Reeves 1994; Vizir et al. 1994; Vollbrecht and Hake 1995). Several genetic approaches to the identification of such haploid-specific genes have been initiated, and some mutations affecting female gametophyte function and the specific step of megagametogenesis have been identified (Birchler and Levin 1991; Springer et al. 1995; Feldmann et al. 1997; Christensen et al. 1998; Howden et al. 1998; Bonhomme et al. 1998; Grini et al. 1999). All of the mutations affecting the development of the megagametophyte described to date affect the early steps of megagametogenesis, and each of the mutant phenotypes is induced by the disruption at a single locus. In contrast, the defective development of the embryo-sac in the *indica-japonica* hybrid is complicated. Unlike the mutations that cause nuclear female infertility (Springer et al. 1995; Ohad et al. 1996; Huang and Sheridan 1996; Feldmann et al. 1997; Chaudhury et al. 1997; Christensen et al.1997; Howden et al. 1998; Grini et al. 1999; Siddiqi et al. 2000), embryo-sac arrest in the hybrid progenies does not occur in their original parents (Yokoo 1984; Liu et al. 1997a). A study of the underlying genetics may shed light on the process of embryo-sac development in angiosperms, and provide important information for rice improvement.

In the investigation presented here, we report direct evidence for female-gamete abortion phenotypes in an *indica-japonica* cross and its derived populations by the analysis of embryo-sac morphology in sectioned ovaries. The abortion phenotype is associated with markers on the rice molecular linkage map, and three major QTLs regulating embryo-sac fertility were identified.

Materials and methods

Plant material

Two *Oryza sativa* cultivars, ZYQ8 (Z) (ssp. *indica*) and JX17 (J) (ssp. *japonica*), were used as parents to produce the inter-subspecific hybrid. A doubled-haploid (DH) population consisting of 132 pure lines was established via anther culture of an F_1 hybrid between ZYQ8 (*indica*) and JX17 (*japonica*) (Lu et al. 1996; He et al. 1998, 1999). All the DH lines in this population were cross-pollinated with the pollens from parental lines ZYQ8 and JX17, respectively, and 202 hybrids were ultimately obtained. The hybrid between ZYQ8 and JX17 was named Z/J hybrid, the hybrids between each of DH lines and ZYQ8 were named the DH/Z hybrids and the hybrids between each of the DH lines and JX17 were named the DH/J hybrids. The plants of the parental cultivars, DH lines and all hybrids were grown in an irrigated paddy field.

Cytological observation

To observe embryo-sac development of the *indica-japonica* hybrid, spikelets of the Z/J hybrid plants at various developmental stages were excised and fixed in FAA fluid (2% formalin + 5% acetic acid + 60% ethanol) and stored in 70% ethanol. After the lemma and palea were removed, the ovaries were imbedded in paraffin and the sections stained in haematoxylin. Cytological observations were made on serial sections of each ovary. The frequency of embryo-sac abortion was determined for every genotype; panicles at the flowering stage were used for the microscope observation, and 50–124 ovaries were checked per genotype.

QTL detection

A molecular linkage map based on the DH population developed in our laboratory was used for quantitative trait locus (QTL) mapping (Lu et al. 1996; He et al. 1999). This map includes a total of 243 RFLP and microsatellite markers distributed over all 12 rice chromosomes. Interval QTL mapping was carried out using the software MAPMAKER/QTL ver. 1.1 with a LOD threshold of 2.0 for declaring the presence of putative QTLs (Lander and Botstein 1989; Lincoln et al. 1993). In addition, the additive effect and the percentage of variation explained by individual QTLs were also estimated.

Results

Embryo-sac development of *indica-japonica* F_1 progeny and a comparison of embryo-sac sterility between the reciprocal F_1 plants

Embryo-sac development in rice follows a typical monosporic-type megasporogenesis combined with a *Polygonum*-type megagametogenesis (Maheshwari 1950; Liu et al. 1995). Differences in embryo-sac development between the Z/J hybrid and parental lines were analyzed using series sections. In general, the parental lines underwent normal megasporogenesis and megagametogenesis. The meiosis of a diploid megasporocyte consists of two successive divisions, which give rise to four haploid megaspores (data not shown). It is the chalazal spore that develops to form the megagametophyte, while the three micropylar megaspores degenerate (Fig. 1A). After first mitosis in the functional megaspore, the daughter nuclei are separated to the micropylar and chalazal poles by a large central vacuole (Fig. 1B) and a second mitosis follows to form four-nucleate embryo-sac (Fig. 1C). The megasporogenesis in the Z/J F_1 hybrid was apparently normal (data not shown). However, in some of the ovaries from the Z/J hybrid plant, megagametogenesis appeared to be blocked at the first round of mitosis (Fig. 1D-F). The degenerating functional megaspore is characterized by no vacuole differentiation, less or withered cytoplasm and a collapsing nucleus. The functional megaspore degeneration resulted in the cessation of female gametophyte development and, as shown in Fig. 1G and H, aborted ovaries without an embryo-sacare visible. In the F_1 hybrid plants of the reciprocal crosses, ZYQ8/JX17 and JX17/ZYQ8, the aborted ovaries without embryo-sac structure accounted for 19.49% and 19.35% of the total ovaries observed, respectively, with

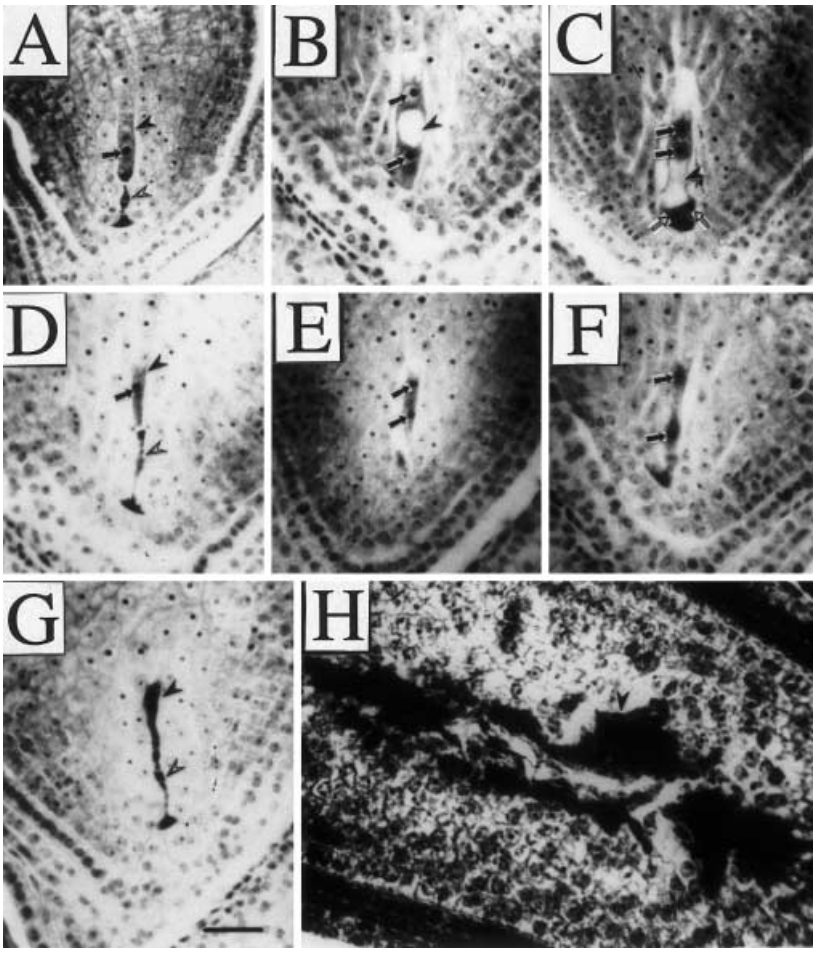

Fig. 1A–H Megagametogenesis in *ssp. indica* cv. ZYQ8 and F_1 hybrids of a cross between *ssp. indica* cv. ZYQ8 and *ssp. japonica* cv. JX17. **A–C** Female gametophyte development of ZYQ8. **A** Developing female gametophyte at one-nucleate stage. The developing female gametophyte [functional megaspore (*filled arrow head*)] is uninucleate (*filled arrow*). The degenerated non-functional megaspores (*open arrowhead*) are shown. **B** Developing female gametophyte at two-nucleate stage. The developing female gametophyte consists of two nuclei (*filled arrow*) which are separated by a large vacuole (*filled arrowhead*) to the micropylar and chalazal poles. **C** Developing female gametophyte at four-nucleate stage. The developing female gametophyte consists of four nuclei. A large central vacuole (*filled arrowhead*) separates a pair of chalazal nuclei (*filled arrow*) from a pair of micropylar nuclei (*open arrow*). **D–H** Defective female gametophyte development in the F_1 hybrids. **D** Degenerating female gametophyte at one-nucleate stage. The degenerating female gametophyte [functional megaspore (*filled arrowhead*)] is uninucleate (*filled arrow*). The degenerated non-functional megaspores (*open arrowhead*) are shown. **E, F** Degenerating female gametophytes at two-nucleate stage. The degenerating female gametophytes consist of two nuclei (*filled arrow*), with less cytoplasm and without vacuole differentiation. **G** Degenerating female gametophyte. The female ganerophyte [functional megaspore (*filled arrowhead*)] was degenerating. The degenerated non-functional megaspores (*open arrowhead*) are shown. **H** Aborted ovary without embryo-sac structure. Degenerated nucellus cells (*filled arrowhead*) around the collapsed embryo-sac are shown. *Bar:* 20μm

Table 1 Embryo-sac fertility of *indica* parent ZYQ8 and *japonica* parent JX17 as well as their reciprocal crosses

Genotypes	Total no. ovaries observed	Number of aborted ovaries observed	Aborted ovaries (%)
ZYQ8(Z)	51	0	0
JX17(J)	58	0	0
ZYQ8/JX17	118	23	19.49
JX17/ZYQ8	124	24	19.35

no significant difference in the frequency of aborted ovaries between the two reciprocal crosses (Table 1). This indicates that cytoplasmic genes have no significant impact on female gametophyte fertility in crosses between ZYQ8 and JX17.

Embryo-sac fertility in DH lines and backcross progenies

From the anther culture of Z/J F_1 hybrid plants, 157 DH plants were obtained, of which 132 developed fully fertile

Table 2 Embryo-sac sterility in backcross populations

Female parent	Male parent: ZYQ 8			Male parent: JX 17		
	Total no. of ovaries observed	Number of aborted ovaries observed	Sterility (%)	Total no. of ovaries observed	Number of aborted ovaries observed	Sterility (%)
DH1	72	7	9.62	75	3	4.0
DH2	52	2	3.85	65	4	6.15
DH4	64	5	7.81	51	1	1.96
DH5	61	7	11.48	52	1	1.92
DH6	71	7	9.80	74	11	14.87
DH7	73	0	0.00	50	1	2.0
DH8	66	19	28.79	71	0	0.0
DH10	57	2	3.51	63	6	9.52
DH11	59	7	11.86	53	3	5.66
DH12	74	19	25.68	60	13	21.67
DH13	58	2	3.45	70	4	5.71
DH14	78	3	3.85	72	23	31.94
DH17	69	4	5.8	52	1	1.92
DH19	51	1	1.96	50	3	6.0
DH20	55	0	0.00	64	5	7.81
DH21	76	13	17.11	67	4	5.97
DH22	62	4	6.45	69	4	5.78
DH23	68	3	4.41	78	3	3.85
DH25	72	0	0.00	79	6	7.59
DH26	59	0	0.00	53	1	1.89
DH32	62	16	25.81	67	3	4.48
DH33	64	1	1.56	69	19	27.53
DH34	77	18	23.38	55	2	3.64
DH36	73	18	24.65	76	8	10.53
DH37	58	9	15.52	54	0	0.0
DH38	54	0	0.00	61	11	18.03
DH40	75	2	16.0	54	1	1.85
DH41	71	10	14.09	52	1	1.92
DH42	75	16	21.33	68	0	0
DH43	76	15	19.74	59	1	1.69
DH44	67	0	0.00	74	0	0.0
DH45	55	2	3.64	57	9	15.78
DH46	51	2	3.92	72	13	18.06
DH47	51	1	1.96	63	9	14.28
DH48	62	0	0.00	50	2	4.0
DH49	78	11	14.10	72	7	9.72
DH50	70	4	5.71	75	12	16.0
DH51	51	7	13.73	64	5	7.81
DH52	62	6	9.68	50	2	4.0
DH53	53	2	3.77	67	16	23.88
DH54	57	0	0.00	75	3	4.0
DH55	56	0	0.00	75	6	8.0
DH56	65	16	24.62	67	13	19.40
DH57	63	1	1.59	63	14	22.22
DH58	71	4	5.63	64	16	25.0
DH59	66	4	6.06	70	7	10.0
DH60	51	3	5.88	54	1	1.85
DH61	71	12	16.90	51	1	1.96
DH62	70	14	20.0	73	0	0.0
DH63	50	2	4.00	73	14	19.17
DH64	51	1	1.96	57	8	14.04
DH65	76	6	7.89	55	3	5.45
DH66	65	12	18.46	64	10	15.63
DH67	59	0	0.00	60	5	8.33
DH68	71	14	19.71	71		18.18
DH69	68	15	22.06	60	13	21.67
DH70	52	2	3.85	70	4	5.71
DH71	69	7	10.15	71	20	28.16
DH72	73	17	23.29	65	4	6.15
DH73	61	7	11.48	58	10	17.24
DH74	60	6	10.0	69	7	10.15
DH75	71	0	0.00	77	3	3.9
DH76	52	2	3.85	57	7	12.28

Table 2 (continued)

Female parent	Male parent: ZYQ 8			Male parent: JX 17		
	Total no. of ovaries observed	Number of aborted ovaries observed	Sterility (%)	Total no. of ovaries observed	Number of aborted ovaries observed	Sterility (%)
DH77	67	13	19.40	69	17	24.64
DH78	77	12	15.58	75	10	13.33
DH79	64	0	0.00	68	11	16.18
DH80	61	10	16.39	58	0	0.0
DH81	55	2	3.64	77	6	7.79
DH82	74	6	8.11	55	15	27.27
DH83	52	2	3.85	58	10	17.24
DH84	67	17	25.37	59	7	11.86
DH85	71	10	14.09	75	3	4.0
DH86	66	20	30.3	62	6	9.68
DH87	63	9	14.29	68	3	4.41
DH88	73	0	0.00	69	16	23.19
DH89	67	4	5.97	54	1	1.85
DH90	64	6	9.38	70	7	10.0
DH94	53	1	1.92	57	8	14.03
DH95	76	14	18.42	52	6	11.53
DH96	79	2	2.53	64	10	15.63
DH97	68	0	0.00	59	15	25.42
DH98	67	12	17.9	54	1	1.85
DH99	59	18	30.51	68	4	5.88
DH101	66	12	18.18	52	1	1.92
DH102	50	2	4.0	68	4	5.88
DH103	76	6	7.89	65	17	26.67
DH104	65	9	13.85	65	4	6.15
DH105	67	13	19.4	54	5	9.26
DH106	62	0	0.0	64	0	0.0
DH107	70	7	10.0	74	0	0.0
DH108	67	11	16.42	59	0	0.0
DH109	68	0	0.00	52	6	11.54
DH112	71	13	18.31	67	18	26.87
DH114	63	4	6.35	66	4	6.06
DH115	62	12	19.36	59	9	15.25
DH116	67	5	7.46	66	8	12.12
DH119	52	0	0.0	61	0	0.0
DH121	50	2	4.0	70	17	24.29
DH122	72	15	20.83	56	9	16.07
DH123	66	22	33.33	72	18	25.0
DH124	62	3	4.84	67	15	22.39

embryo-sacs; 25 failed to produce viable embryo-sacs due to the functional megaspore degeneration. Since genotypes of the DH lines represent haploid genotypes of the pollens from the Z/J F_1 hybrid in this study, crosses between each of the DH lines and a parental line are genetically identical to the backcross of the Z/J F_1 hybrid to the parental line. Thus each of 132 DH lines was then backcrossed to the *indica* parent, ZYQ8, and *japonica* parent, JX17, respectively, and ultimately 101 DH/Z hybrids and 101 DH/J hybrids were obtained. By using series sections, we were able to cytologically evaluate the embryo-sac fertilities of all hybrids from the DH/Z and DH/J crosses. The results showed that the embryo-sac sterility segregated in both DH/Z and DH/J populations (Table 2; Fig. 2A, B). In the DH/Z hybrid population, the percentage of aborted embryo-sacs varied from 0.0% to 33.33%, with a mean of 10.23%, while in the DH/J hybrid population it varied from 0.0% to 31.94%, with a mean of 10.53%. Some DH lines (Table 2) appeared to produce fully fertile embryo-sac hybrids when crossed to either *indica* parent ZYQ8 or *japonica* parent JX17 (Table 2).

Identification of major QTLs for embryo-sac sterility

As described above, all of the DH lines which were used for the genetic map construction were able to produce fully fertile embryo-sacs, whereas the DH plant with a completely aborted embryo-sac could not form a reproductive line. Therefore, the DH mapping population itself is not capable of mapping the loci affecting embryo-sac fertility due to a lack of phenotypic variation. Fortunately, segregation for embryo-sac fertility exists in both populations of the DH/Z and DH/J hybrids (Table 2; Fig. 2A, B), which allowed us to locate the relevant loci. Moreover, diploid F_1 hybrids from both DH/Z and DH/J

Table 3 QTLs detected for embryo-sac abortion based on interval mapping in DH/Z and DH/J populations. LOD, log-likelihood; PVE, percent phenotypic variation

Mapping population	Locus	Marker internal	Chromosome	Interval length (cM)	LOD score	PVE	Phenotypic effect
DH/Z	*esa-1*	G200-C235	6	13.8	5.02	24.2	+9.02
DH/J	*esa-2*	RG181-G1106	12	10.4	4.20	20.9	+7.97
DH/J	*esa-3*	RG323-RG463	12	19.7	4.42	24.0	+8.48

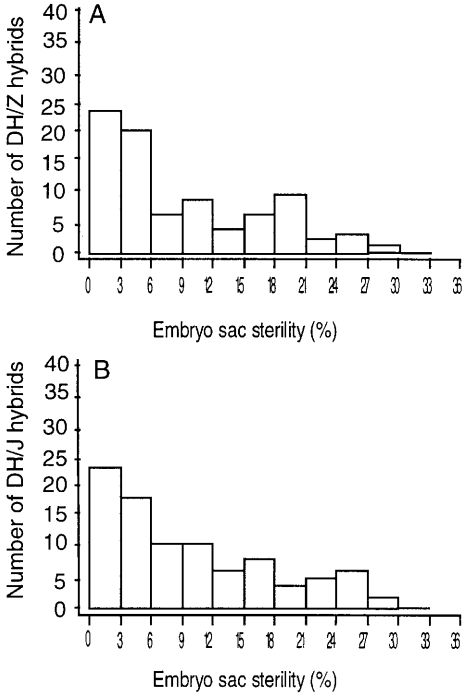

Fig. 2A, B Distribution of embryo-sac sterility in: **A** hybrids between DHlines and ZYQ8 (DH/Z hybrids); **B** hybrids between double haploid lines and JX17 (DH/J hybrids)

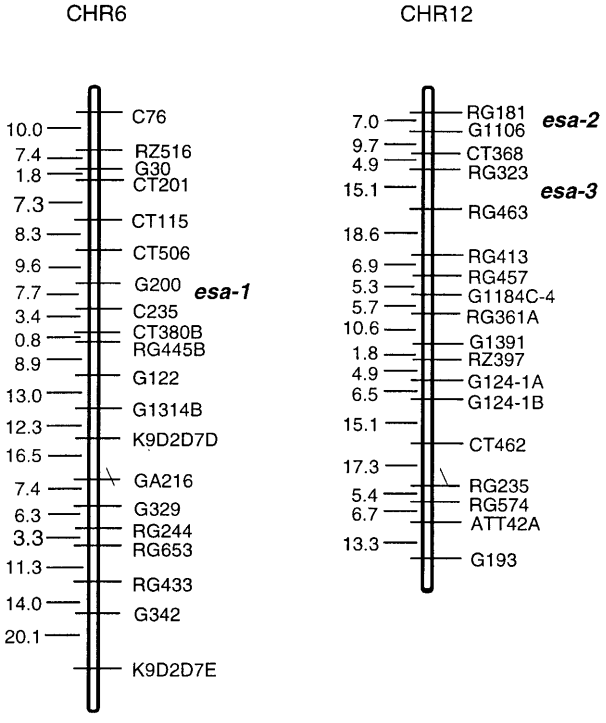

Fig. 3 Genetic map of rice chromosomes 6 and 12 showing the locations of *esa-1* (embryo-sac abortion), *esa-2* and *esa-3*. A rice linkage map and backcross progeny testing were used to identify these three loci that are involved in regulating embryo-sac fertility in an *indica-japonica* cross. The map was constructed using a DH population consisting of 132 DH lines and a total of 243 RFLP and microsatellite markers distributed over all 12 rice chromosomes. Molecular markers are indicated to the *right* of each chromosome and the genetic distances in Kosambi centiMorgans (cM) are shown on the *left*. The major loci for the embryo-sac abortion are shown on the *right* of chromosome in *bold italics*

crosses consist of two sets of genomes, one originating from one of the parental lines while the other from each of the DH lines. The first set of genomes in either the DH/Z or DH/J hybrid population is invariable and carries the same parental genotype for embryo-sac fertility in the respective population because the same parental line participated in all of the DH/Z or DH/J crosses. However, the second set of genomes in both DH/Z and DH/J hybrid crosses varies with different donors of the DH lines. Consequently, the phenotypic variation in embryo-sac fertility in the DH/Z and DH/J hybrid populations is determined by the genomes of the DH lines. We were therefore able to use the RFLP linkage map generated from the DH population to locate QTLs accounting for phenotypic segregation of embryo-sac fertilities in the DH/Z and DH/J hybrid populations. By interval-QTLs mapping, three loci with major effects, one in the DH/Z population and the other two in the DH/J population, were identified (Table 3). The QTL detected in the DH/Z population, named *esa-1* (embryo-sac abortion), is bordered by markers G200 and C235 on chromosome 6, while the QTLs detected in the DH/J population, named *esa-2* and *esa-3*, are bordered by markers RG181 and G1106 and RG323 and RG463, respectively, on chromosome 12 (Fig. 3). The three loci, *esa-1*, *esa-2* and *esa-3*, could explain 24.2%, 20.9% and 24.0% of the total phenotypic variation (Table 3).

Discussion

Embryo-sac development takes place inside the ovule. Genetic analysis has yielded a number of sporophytic mutants defective in ovule development, embryo-sac development, or both (Kennel and Horner 1986; Robinson-Beers et al. 1992; Jofuku et al. 1994; Leon-Kloosterziel et al. 1994; Gaiser et al. 1995; Hulskamp et al. 1995; Elliott et al. 1996; Klucher et al. 1996; Byzova et al. 1999). The phenotypes of the previously described megagametophytic mutants suggest an early requirement for haploid-expressed genes (Redei 1965; Simcox et al. 1986; Liu et al. 1995; Ohad et al. 1996; Huang and Sheridan 1996; Christensen et al. 1997; Moore et al. 1997; Drews et al. 1998; Grossniklaus and Schneitz 1998; Howden et al. 1998; Siddiqi et al. 2000). An elaborate study in maize based on a systematic generation and characterization of chromosome deletions unraveled three different processes that can be disrupted by mutation: progression through nuclear stages, synchronization of events and establishment of cellular patterns (Vollbrecht and Hake 1995). In our study, the phenotype of defective female gametophyte development is more or less similar to that of the reported mutants which appeared to be impaired in the early steps of embryo-sac development (Feldmann et al. 1997; Christensen et al. 1998; Howden et al. 1998; Bonhomme et al. 1998; Grini et al. 1999). According to our observations, embryo-sac abortion was manifested following meiosis. When the surviving functional megaspores began to wither, the nucleus either failed to divide or divided only once. This failure of early megagametogenesis may therefore specifically affect a function that is required in the functional megaspore precursor for mitosis.

Extensive investigations into the genetic basis of post-zygotic reproductive barriers among varieties of *O. sativa* have provided evidence that even among closely related materials, multiple pathways can lead to reproductive isolation. Hybrid sterility due to chromosomal aberrations has been suggested (Henderson et al. 1958; Yao et al. 1958; Shastry and Misra 1961; Delores et al. 1975), but it could not, in the majority of cases, be attributed to cytologically detectable abnormalities (Chu et al. 1969). Several lines of evidence have indicated the involvement of major genes in rice hybrid sterility in which alleles at a single locus or two complementary loci could either cause sterility or recover fertility in the hybrids (Kitamura 1962; Oka 1974, 1988; Ikehashi and Araki 1986; Li et al. 1997). Certain rice varieties, called ''wide compatibility varieties'', have been reported to produce fertile F_1 hybrids when crossed to either ssp. *indica* or ssp. *japonica* varieties (Ikehashi and Araki 1986). Hybrid sterility also appears to be due to a recombination within a number of putative differentiated supergenes in the rice genome (Li et al. 1997). In addition, the cytoplasmic genome has been found to have a large effect on hybrid fertility (Chang et al. 1990; Pham 1990; Li et al. 1997). However, these hypotheses have been proposed largely on the basis of pollen fertility and/or seed set rate. Our study, which was dependent on the direct phenotyping of female gametophyte morphology and QTL mapping, suggested that a polygenic system may also influence a single event in the process of early megagametogenesis as well as post-zygotic isolation in rice. Although in the present study we were unable to explain how these loci interact and regulate embryo-sac fertility, we did find some clues for an interaction of complementary genes that behaved like wide-compatibility genes in the Z/J, DH/Z and DH/J crosses. In fact, we found some DH lines (such as DH44, DH106) in the DH population that manifested wide compatibility to produce embryo-sac-fertile hybrids when crossed to either of the *indica* or *japonica* parents (Table 2). The lethal duplicate recessive nature of the putative complementary gene loci is also suggested by the observations that embryo-sac abortion in the Z/J hybrids did not occur in their original parents, and that in F_1 hybrid plants of the Z/J cross, fewer than one-fourth of the female gametophytes were aborted (Table 1). An interesting phenomenon observed from the doubled-haploid study is that the completely sterile individuals (presumable the duplicate recessive homozygotes) are otherwise viable and normal, suggesting that these defective modifications affecting female gametophyte development do not affect sporophyte development. These observations are in agreement with many other studies: genetic mapping studies of experimental hybrids indicate that most post-zygotic reproductive barriers in plants are polygenic and that the expression of extreme or novel traits in segregating hybrids results from the complementary action of divergent parental alleles (reviewed by Rieseberg et al. 2000).

The wide range in embryo-sac sterility in the DH/J and DH/Z crosses could be attributed to the nature of multiple loci. Nevertheless, only 24.2% of the total variance is explained by *esa-1* in the DH/Z population, while 44.9% is explained by *esa-2* and *esa-3* together in the DH/J population. One possible reason for this is that new mutations arising during anther culture and then being fixed in the DH lines could modify embryo-sac fertility but not be genetically detectable by QTL mapping in these populations. Another possibility is the partial penetration of female gametophyte-specific gene action, as observed in the mutations affecting early megagametogenesis in *Arabidopsis* (Howden et al. 1998; Grini et al. 1999). The third possible reason is the relatively small sample size, which might influence the accuracy of phenotyping, although tremendous efforts were made during the tedious sectioning. An alternative is to utilize clarification techniques in order to simplify the analysis of the embryo-sac development and to obtain more precise information regarding the phenomenon (Stelly 1984).

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