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Advances in Agronomy, Plant Breeding and Horticulture

Full Length Research Paper

Vol. 1 (3), pp. 39 - 43, June, 2013

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Examination of the inheritance of fertility restoration of rice WA CMS in normal and cold conditions

Sani Ahmadikhah and Umaru A. M

Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract

The inheritance of pollen fertility restoration in 2 F_2 populations of cross Neda-A X IR36, was studied in normal and cold conditions by means of QTL method. In cold condition the number of fertile plants was reduced in favor of sterility. The distributions of individuals in pollen fertility suggested that fertility restoration was mainly controlled by major genes. The population cultivated in normal conditions showed a 15 (F): 1(S) ratio; however, the one cultivated in cold condition deviated from 15 (F): 1(S) ratio, in which 2 QTLs were detected. The obtained results showed that one major QTL (qRf- 1-1) localized on the short arm of 1st chromosome near RFLP marker RG140 and the other one (qRf-1-2) localized on the same chromosome between RM7180 and RM6100d. The detected QTLs explained 62 and 44% of the total variation of the trait, respectively. The minor QTL (qFer1-2) is expected to be a cold - inducible modifier QTL for fertility restoration.

Keywords: Rice, fertility restoration, cold condition, QTL.

INTRODUCTION

Most plant traits are quantitative in nature and are influenced by many genes or quantitative trait loci (QTLs). Quantitative traits are also influenced by the en-vironment and tend to show varied degrees of genotype × environment interactions (GEIs). GEIs occur when two or more genotypes perform differently in different environments, and are thus described as differential genotypic sensitivities to environments (Falconer, 1981). Plants, particularly self-pollinated plants, tend to show a high level of GEIs that allow better adaptation to their changing environments and the maintenance of genetic variation in populations (Jain and Marshall, 1967) . In plant breeding, GEIs must be considered to identify superior and stable genotypes when breeding materials are tested in different environments. Because of their importance in plant breeding and evolution, GEIs of quantitative traits have been the subject of extensive investigations (Baker, 1988; Cooper and Hammer, 1996). Classical studies on GEIs using segregating plant popu-lations have been few, but they have yielded valuable information regarding the importance of GEIs for quantitative traits (Mather and Jinks, 1982).

DNA markers and high-density genetic maps of major crops developed since the late 1980s have facilitated effort to understand the genetic basis of quantitative traits through QTL mapping. Main-effect QTLs (M-QTLs) affecting a wide range of agronomic traits in many plant species have been reported (Paterson, 1995; Georges, 1997; Stuber, 1997).

Cytoplasmic male sterility (CMS) is a maternally inherited condition in which sterile plants unable to produce functional pollen. CMS is commonly found in natural plant populations and is most frequently caused by chimerical mitochondrial genes (Schnable and Wise, 1998). In response to CMS, in many populations, nuclear restorer genes have evolved. Searching for restorer genes is a good example where phenotyping is very time-consuming and requires determination of spikelet sterility in testcross progeny (Ahmadikhah and Karlov, 2006). A single restorer gene, or the concerted action of several major and minor restorer genes, may be able to completely restore the fertility of a male sterile individual plant. The molecular mechanisms for CMS and for restoration differ among species and there are a large number of different restoration systems (Schnable and Wise, 1998).

Today, the rice chromosome maps containing molecular markers (McCouch et al., 2002) are accessible. By the use of molecular markers it was possible to create a

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"consensus" map of the rice chromosomes (Kishimoto et al., 1993), integrated from morphological and molecular loci. A large number of works in the mapping of genes in rice have been devoted to the study of the fertility restoration using molecular markers (Zhang et al., 1995, 1997, 2002; Zhuang et al., 2001, 2002; He et al., 2002; Jing et al., 2001; Yao et al., 1997), but because of the complexity of the phenotypic expression, presence of several CMS systems and involvement of many genes and modifiers in the expression of the trait, the fertility restoration genes, especially for WA CMS have not been exactly identified and localized.

The objective of this work was to study the inheritance of fertility restoration of rice WA CMS in normal and cold conditions by means of molecular markers.

MATERIAL AND METHODS

The experimental population and field trials

In the first experiment, the mapping population comprised of 209 F_2 plants derived from the cross between 2 *indica* varieties, Neda A and IR36. The line IR36 is a restorer line carrying Rf3 gene (Ahmadikhah et al., 2005). The mapping population for second experiment comprised of 148 F_2 plants derived from the cross between these 2 *indica* varieties. The first population was grown in the field of Agriculture faculty of Gorgan University of Agricultural Sciences and Natural resources (Northern Iran) and the second was grown in greenhouse of the selection station of Russian State Agrarian University (Moscow, Russia). At flowering stage, the pollen fertility was assayed using 1% I2-KI solution under light microscope.

Data analyses and PCR conditions

DNA was extracted by the use of CTAB method (Saghai-Maroof et al., 1984) with a few modifications. A total of 46 markers, including 39 SSRs, 5 RFLP-based primers selected from the published rice map (McCouch et al., 2002) and 2 morphological traits (plant height = PH and purple stigma = PS), was used to construct a linkage map for the F_2 population. The above mentioned markers were selected based on the earlier reported QTL locations for fertility restoration of WA CMS to decrease the costs and save the time needed for searching promising molecular markers (Zhang et al., 1995, 1997, 2002; Zhuang et al., 2001, 2002; He et al., 2002; Jing et al., 2001; Yao et al., 1997).

PCR reactions were performed in 20 µl volumes containing 0.2 µM/l of each primer, 200 µM/l dNTPs, 50 mM/l KCl, 10 mM/l Tris-HCl, 1.5 mM/$MgCl_2$ and 1 unit of Taq DNA polymerase. The PCR profile was 94°C for 5 min (denaturation), followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and finally 72°C for 7 min in the final extension. The products from PCR reaction were resolved by electrophoresis in 2% agarose gel containing 0.5 µg/ml ethidum bromide.

Linkage map construction and QTL analysis

JoinMap software (Stam, 1995) was used to calculate the marker distances and to assign the linked markers to linkage groups. QTL Cartographer 2.0 (Wang et al., 2005) was used for QTL analysis. Correlation between the polymorphic markers and the trait was performed by single marker mapping. QTL parameters (locations, effects and test statistics) of all putative main QTL (M-QTL) and QTL interactions (I-QTL) pairs were estimated using the multiple interval mapping (MIM) and the restricted maximum-likelihood estimation method with all those markers identified in the first step was fixed in the model to control the background genetic variation. The LOD > 2.5 was used for claiming significant M-QTLs and interactions in this study.

RESULTS AND DISCUSSION

The average of pollen fertility of F_2 population grown in normal conditions was 73.6 ± 2.26%, but that of F_2 population grown in cold condition in the greenhouse of selection station of RSAU, Moscow was 49.3 ± 5.34%. These differences indicate the differential responses of F_2 plants in different conditions, with decreasing the average pollen fertility in stressful condition.

The F_2 population cultivated in normal conditions was segregated to 202 fertile (96.6% of total F_2 plants) and 7 sterile (3.4% of total F_2 plants) plants, which agrees with 15:1 ratio (^{2} = 3.0, $^2_{05}$ = 3.84), indicating involvement of 2 gene in the control of fertility restoration in normal condition (Table 1, Figure 1).

However, an abnormal segregation was observed in F_2 population grown in stressful condition. On the basis of pollen fertility test, 4 classes of plants were distinguished:

1) 56 sterile plants (pollen fertility < 10%).
2) 27 partial sterile plants (pollen fertility 10 - 70%).
3) 18 partial fertile plants (pollen fertility 70 - 85%).
4) 47 completely fertile plants (pollen fertility > 85%) (Figure 1).

Taking into account 3 later classes as fertile class, the F_2 population was segregated to 92 (62.2% of total F_2 plants) fertile and 56 (37.8% of total F_2 plants) sterile plants, which deviates significantly from 3:1 ratio (^{2} = 13.01, $^2_{05}$= 3.84) or from15:1 ratio (^{2} = 252.03; $^2_{05}$ = 3.84) and well agrees to 9:7 ratio (^{2} = 2.10, $^2_{05}$ = 3.84), indicating involvement of 2 genes interacting in a complementary manner in the stressful conditions. In earlier studies, involvement of 2 genes in the control of fertility restoration, interacting complementarily with each other (9F : 7S) has been reported (GovindaRaj and Siddiq, 1984). Maekawa (1980) also reported such type of action of fertility restoration genes Rf-a, Rf-b and Rf-c.

Detection of QTL loci with main effects (M-QTLs) in cold condition

Molecular markers RM171, RM3773, RM311, RM6100, RM6128 and AB443 (on 10-th chromosome), RM1, RG140 and RM7180 (on 1-st chromosome) and RM1335, RM1209 and RM3555 (on 7-th chromosome) have shown polymorphism between Neda- A and IR36. The QTL analysis was carried out with 12 chosen polymorphic molecular and 2 morphological markers (PH = plant height and PS-1 = stigma color).

Using single marker mapping analysis, 4 markers on 1-st chromosome (3 molecular and 1 morphological) have

Table 1. Distribution of frequency of pollen fertility of F2 population grown in 2 conditions.

	Pollen fertility (%)							
F_2 population grown in…	**< 10**	**% plants of total**	**10 - 70**	**% plants of total**	**70 - 85**	**% plants of total**	**> 85**	**% plants of total**
Normal conditions	7	3.35	25	11.96	87	41.63	90	43.06
Stressful conditions	56	37.84	27	18.24	18	12.16	47	31.76

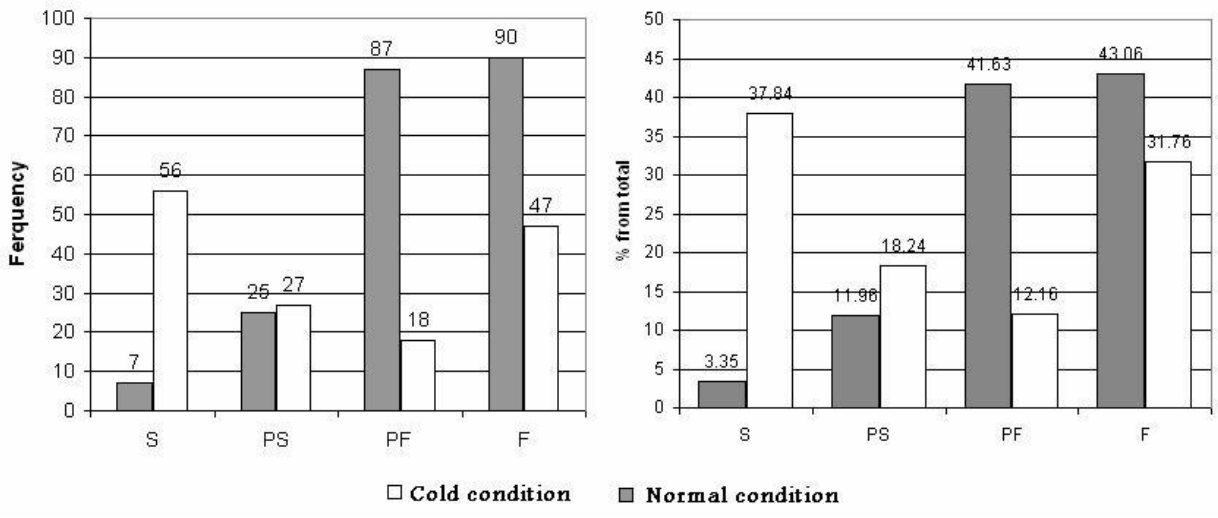

Figure 1. Distributions of pollen fertility of the F_2 plants in normal and cold conditions in terms of frequency (left) and percent of each class (right).

Table 2. The parameters obtained by I method for each QTL locus.

QTL	Closest marker	LOD	QTL Pos.	Len	Ve (%)	Effect				d/a
						a	d	a (%)	d (%)	
qFer1-1	RG140	18.85	10	3.90	61.9	40.47	4.87	62.1	5.6	0.12
qFer1-2	RM7180	2.96	36	4.54	43.8	-19.20	57.44	-11.9	38.2	-2.99
Total		21.81						93.97		

Pos = QTL position; Len = QTL length; Ve = the percent of the total variability causing by a QTL; a = additive; d = dominance; d/a = the degree of dominance.

have been detected, having a correlation with the fertility restoration (Table 2). Among them, RM1 and RG140 had very high correlation (P > 0.9999) and RM7180 and PH had intermediate correlation (P> 0.99) with the trait. The linkage map for the 1-st rice chromosome, consisting of 4 DNA markers (3 SSR and 1 RFLP) and 2 morphological markers covering ~95 cM (Figure 2) has been created. In normal conditions 1 major gene (Rf3 in Figure 2) was detected and mapped on the short arm of rice chromosome 1.

However, For QTL analysis of the trait in stressful conditions the multiple interval mapping (MIM) method was used by means of WinQTL Cartographer 2.5 program (Wang et al., 2005). 2 main QTL loci (-QTLs) were detected for fertility restoration in the investigated population with high enough LOD thresholds. The first QTL (qFer1 - 1) with LOD > 18 was close to RG140 marker and the second (qFer1-2) with rather low LOD threshold (LOD = 2.96) was close to RM7180 on 1-st chromosome (Table 2, Figure 2). These 2 -QTLs, respectively, are limited to the intervals between markers RG140 - RM7180/PH and RM7180/PH - RM6100d, with the QTL lengths of 3.90 and 4.54 cM.

Multiple interval mapping has allowed us to calculate genetic parameters of the trait. As shown in Table 3, qFer1-1 causes ~62% and qFer1- 2 ~44% from the total variability of the trait. qFer1-1 has higher additive effect, than its dominance effect. On the contrary, qFer1-2 has

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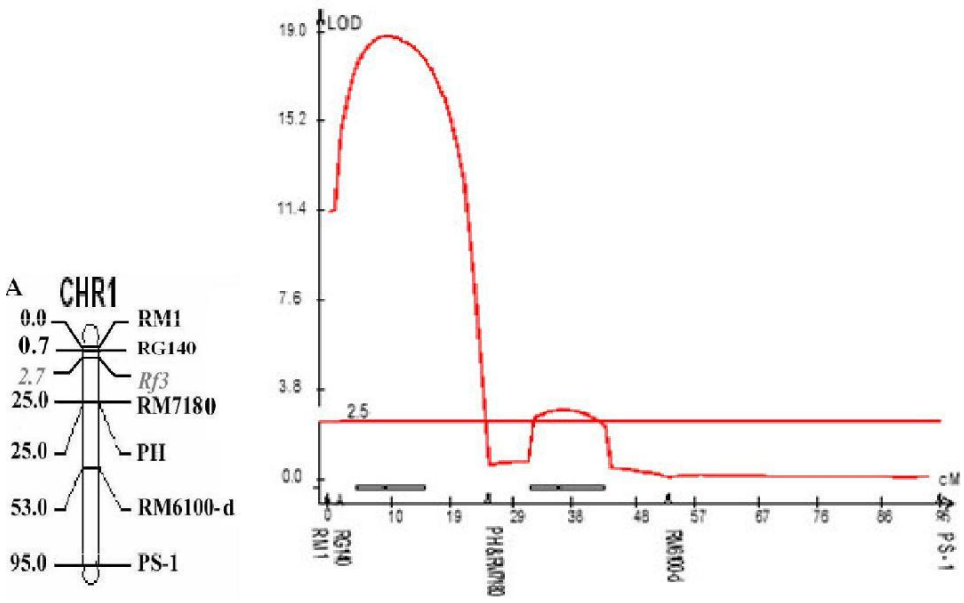

Figure 2. The graphic results of QTL on single markers for construction of the linkage map of markers (left), and for detection of the most probable QTL loci causing fertility restoration (right).

Table 3. Parameters of the detected QTL loci after carrying out of the analysis of interaction.

QTL	Closer marker	LOD		Position	Effect (%)	
		a	d		a	d
qFer1-1	RG140	2.33	0.19	10	77.02	3.31
qFer1-2	RM7180	0.06	2.58	36	-5.94	19.54
Total		5.16			93.93	
iAA					20.88	

iAA- additive by additive interaction.

relatively average negative additive effect and high dominance effect on the trait, which specifies action of the QTL as over-dominance in an opposite direction of the first QTL. As a whole, these 2 QTLs cause ~94% from the total variability of the trait.

The analysis of interaction between the detected QTL loci (I-QTLs)

MIM method has allowed revealing the interaction between the detected QTL loci. After comparison of the LOD thresholds obtained from association of QTLs together (5.16 in Table 3) with total value of individual LOD thresholds (18.85 + 2.96 = 21.81 in Table 3) it is possible to conclude that the epistatic interactions exist between the detected QTL loci.

Results of MIM have showed that the 2 detected QTLs have significant additive by additive interaction with each other (Table 3). This interaction is resulted in the significant modification of the additive effects of 2 QTL loci in stressful conditions, so that the number of sterile plants in F_2 population increases (from 3.35 in normal conditions to 37.84% in stressful conditions). Comparison of percent of plants in each class in 2 conditions show that this interac-tion not only affected the sterile class, but also increased the frequency of plants in partial sterile class (from 11.96 to 18.24%) and finally resulted in the reduction of number of plants in fertile classes (partial fertile, PF and fully fertile, F classes; Figure 1) in favor of non- or low-fertile plants (from 41.63 to 12.16% and from 43.06 to 31.76%, respectively). In fact, existence of these 2 detected QTL loci interacting with each other, explains the deviation of inheritance of the trait from Mendlian 15:1 ratio in stressful cold condition.

Our finding of major QTL for fertility restoration, provide support for the view that phenotypic differences between plants often may be controlled by QTLs of large effect. The reported results of essentially all QTL mapping experiments are consistent with the oligogenic model and not with the infinitesimal model (Paterson et al., 1988; Stuber

et al., 1992; Doebley and Stec, 1993; Bradshaw et al., 1995). That is, much of the phenotypic variance in many traits appears to be under the control of one or a few major QTLs, perhaps modified by QTLs of minor effect. Often it is not clear whether this general outcome is a true reflection of the underlying genetics or a statistical artifact caused by sampling bias (Beavis, 1994; Beavis, 1998), incorrect specification of the mathematical model for QTL detection (Visscher et al., 1996), or some other methodological problem.

Since the effect of first major QTL (qFer1-1) was modified due to the interaction between 2 QTLs, the minor QTL locus (qFer1-2) detected in this study is expected to be a cold-inducible modifier QTL for the fertility restoration. Appearance of the partial sterile and fertile plants in crossing of strong restorer lines (in this study IR36) with CMS lines (existence of 27 and 18 plants, respectively, as shown in Figure 1), indicates the probable role of modifier genes in the occurrence of fertility restoration as suggested by Kumari (1998) that are activated in desirable environmental conditions.

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