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Genetic analysis for cold tolerance at booting stage for *japonica* rice (*Oryza sativa* L.)

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Abstract

'Kunmingxiaobaigu' rice (*Oryza sativa* L.) has two major genes effectively manipulating the grain fertility. Four cultivars (Lijing 2, Xintuanheigu, Xiaomagu and Diahjing 8) have one or two loci of major gene manipulating the grain fertility. There are some difference on cold tolerance between Kunmingxiaobaigu and Lijing 2. The gene for cold tolerance at booting stage was further mapped on chromosome 5 between RM 7452 and RM 31 with genetic distances of 4.8 cM and 8.0 cM, respectively. This gene explained 10.50% of phenotypic variance and 5.10% of phenotypic variance of fully filled grains and was tentatively designated as *Ctb*(t).

Key words: Rice, genetic analysis, cold tolerance, booting stage

Introduction

Cold damage in rice (*Oryza sativa* L.) is a common problem in its cultivation all over the world especially in Japan, Korea and China. It is, however, a major problem in Yunnan [1]. Yunnan is a centre of ecological genetic diversity for cold-tolerant rice resources, existing under various ecosystems, and is most advantageous for the evaluation and breeding of near-isogenic lines (NILs) for cold-tolerance [2]. Cold tolerance in rice is a quantitative character up to 7 genes controlling the grain fertility [1]. Due to difficulty in precise location and cloning of cold tolerance genes in chromosome, we seek major genes and develop NILs for cold tolerance at booting stage in rice. We have identified genes for cold tolerance at booting stage in rice through line selection, under the natural and controlled temperature conditions backcrossing and developed the NILs for cold tolerance [1].

Materials and methods

Fourteen crosses and backcrosses from five standard rice cultivars (Kunmingxiaobagu, RR; Dalizaoxian S; Lijing 2, RR; Toawda, SS; Hexi 35, M), or 21 crosses from seven rice cultivars with different grades of cold tolerance involving *nuda* (Haomei, Haobi), *javanica* (Sanpangqishilou) and *communis* ('02428', 'Kunmingxiaobaigu', 'Lijing 2', 'Banjiemang') with different grades of cold tolerance were used for the genetic analysis of F_1 and F_2 in cold tolerance characters using the method put forward by Griffing II [3]. Genetic analysis between 02428 (SS) or Diannong S-1 of the least (SS) and 6 of the strongest (RR) cold tolerance at booting stage were carried out. Five pairs Chinese NILs for cold tolerance at booting stage in *japonica* rice based on line selection and backcrossing and artificial mutagenesis have been developed. A BC_5F_2 population consisting of 154 individuals was developed from a cross between NIL (BC_5F_2) and Towads (recurrent parent) based on 3 markers with polymorphism among parents from 164 SSR markers.

Results and discussion

Genetic analysis on cold tolerance at booting stage in rice: Genetic analysis of cold-tolerance under warm-cool region of *japonica* rice was carried out using 15 crosses (including NILs) and 5 parents from different cold-tolerant rice. The size and GCA on characteristic of less cold tolerant varieties is smaller and lower than cold tolerant varieties, and there is great difference in parent's GCA and SCA estimated from different generations, which is related with predominance declining and stress selecting [3]. 'Kunmingxiaobaigu' has two major genes effectively manipulating the grain fertility; 'Lijing 2' has one or two loci of major gene manipulating the grain fertility, while gene expression of their cold tolerance is related with cytoplasm effect. Main peaks of the frequency distribution of F_2 or F_3 individual plant fertility between 'Dalizaoxian' and 'Lijing 2', 'Towada', 'Hexi 4' were located at 10%~20%, but main peaks of fertility distribution of 'Kunmingxiaobaigu × Dalizaoxian' at 60%-80% [4].

Yunnan local rice consists of six ecogroups of two subspecies. *Javanica, nuda* and *communis* of Yunnan *japonica* subspecies account for 3.6%, 18.1% and 32.1%, respectively; Early-mid *indica*, late *indica* and winter *indica* of *indica* subspecies account for 2.1%, 43.9% and 0.2%, respectively [5]. There are clear differences on cold tolerance genes between of *javanica* ('Sanpangqishiluo') and *nuda* ('Haomei', 'Haobi') and *communis* ecogroups ('02428', 'Lijing 2', 'Kunmingxiaobaigu', 'Banjiemang'), and these cultivars including *javanica* and *nuda* and *communis* ecogroups identified on cold-cool *japonica* region in Yunnan Province are weak in cold-tolerance, but differences

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could be found [6]. In the dominant-recessive relationship, gene number and effect, there are distinct differences between various ecogroups with good cold tolerant cultivars (Kunmingxiaobaigu' and 'Lijing 2' and 'Banjiemang'). The cold tolerance of *japonica* subspecies is divided into four types i.e. *javanica, nuda*, subtropical plateau and temperate plain *javanica* rice.

By utilizing '02428' with the least cold tolerance at booting stage (1.0%) as the genetic background, it is inferred that the cold tolerance of 'Xiaomagu' (seed setting rate 91.5%) and 'Xintuanheigu' (93.3%) is controlled by one or two loci of major gene for grain fertility under cold condition [1]. Cold tolerance of 'Kunmingxiaobaigu' and 'Jingdiao 3' is controlled by one loci major gene and poly gene manipulating the grain fertility, respectively, by using 'Diannong S-1' of TGMS as the background. 'Diannong S-1 × Banjiemang' F_1 has the highest heterosis, capable of increasing the yield by 50% in contrast with the good cultivar 'Hexi 35' [1]. There are at least 5 loci to control the fertility expression of F_2 segregated populations. F_2 segregated populations of 'Diannong S-1 × Dianjing 8' showed that there is one-loci major gene manipulating fertility expression. There are significant differences among cultivars with strongest cold tolerance such as cultivars 'Dianjing 8' and 'Kunmingxiabaigu', 'Jingdiao 3' and 'Banjiemang'.

Development of Chinese NILs for cold tolerance at booting stage in japonica rice: Cold tolerance at booting stage in rice is a complex quantitative character. We have established the system of evaluation for cold tolerance at booting stage in *japonica* rice. For example, Kunming (1916 m amsl) is a rice-growing region where low temperatures frequently occur and good results could be achieved at this place. However, characterization under natural conditions is affected by varietal differences in maturity date and uncertainty in low temperature occurrence [2]. Consequently, adoption of controlled temperature methods (e.g. cold-water pool and constant temperature method) has been shown to give better results, but the amount of material treated is limited and the cost is high. A simple and convenient method is the long-period cold-water irrigation at Aziying (2150 m amsl) which is an ideal location for cold tolerance evaluation, because there water temperature is 17~18°C from a cool-spring for irrigation under natural condition.

NILs for cold tolerance at booting stage from line selection: 'Xinan 175' was the most important variety grown on Yungui plateau in 1960-80. The area planted to 'Xinan 175' in 1982 was nearly 1 million ha. It is very rich in variation type, high in yield, but poor in cold-tolerance. Through natural and artificial selection of Yunnan rice from five regions over forty years (1958-1998), the following cold-tolerant 9 cultivars for difference cold tolerance were selected: '65-113' (lowest), '129' (lowest), '65-36' (low), '78-251' (middle), '331' (middle), '251 Select' (middle)[1], '175 Select 3' (High), '75-64' (High) and 'Yunjing 9' (Highest) [7]. In these varieties, cold tolerance is connected with the breeding locality; NILs.can be identified from these varieties. 'Xinan 175' (S) and 'Yunjing 9' (R) are used as the check varieties [2, 7]. 'Yunjing 9' of stronger cold tolerance was selected from 'Xinan 175' of weak cold tolerance by line selection. F_2 segregated populations of 'Xinan 175 × Yunjing 9' showed that cold tolerance for Yunjing 9 is controlled by poly gene [1]. The hybrids acclimation for 2 to 3 years could be obtained and strengthened cold tolerance at booting stage [8]. Therefore, cold acclimation in different rice regions in Yunnan Province can be used to breed rice NILs with cold tolerance at booting stage.

NILs for cold tolerance at booting stage from backcrossing: The rice variety, 'Towada' (seed setting rate, 17.2%), is the least cold toleranct variety. A set of NILs or QTL-NILs of *japonica* rice with major genes or polygene for cold tolerance at booting stage were developed from 1994 to 2000, using 'Towada' as recurrent parent, and 'Kunmingxiao-baigu' (seed setting rate, 88.3%), 'Banjiemang' (88.3%) and 'Jingdiao 3' (87.2%), which are best in cold tolerance at booting stage, as donor parents. The agronomic characters of NILs or QTL-NILs are similar to 'Towada' as recurrent parent, but there are clear differences in seed setting rate for cold tolerance, i.e., seed setting rates of these NILs are over 60% higher than that of 'Towada' (17.2%). In cross between Towada and Banjiemang, the seed setting rates of the following NILs are higher than that of Towada: 'Towada^5/Banjiemang' (BC_4F_6, 95.1%), 'Towada^6/Banjiemang' (BC_5F_4, 95.0%; BC_5F_3, 93.1%), 'Towada^7/Banjiemang' (BC_6F_3, 96.6%; BC_6F_2, 91.3%). The distribution of the seed setting rate for 142 individuals of 'Towada^8/Banjiemang' BC_7F_2 showed the one-peak normal distribution, located in 31%-50%, with seed setting rate ranging from 8.4% to 88.5%, i.e., this NIL belongs to QTL-NILs. In cross between Towada and Jingdiao 3, the seed setting rate: Towada^4/Jingdiao 3' (BC_3F_5, 91.1%), Towada^5/Jingdiao 3' (BC_4F_4, 75.8%; BC_4F_3, 81.9%), Towada^6/Jingdiao 3' (B_5CF_3, 73.8%) are also higher than that of 'Towada'. The distribution of the seed setting rate for 149 individuals of 'Towada /Jingdiao 3' BC_6F_2 showed the one-peak normal distribution, located in 41%-50%, with seed setting rate ranging from 7.2% to 91.3%, i.e., this NIL also belongs to QTL-NILs.

The seed setting rates in cross between Towada and Kunmingxiaobaigu are 'Towada^4/Kun-mingxiaobaigu' (BC_3F_7, 81.7%; BC_3F_6, 92.2%; BC_3F_5, 93.0%), BC_4F_3 (87.8%), 'Towada^5/Kunming-xiaobaigu' (BC_4F_4, 94.8%; BC_4F_3, 87.8%; BC_4F_2, 85.8%), Towada^6/ Kunmingxiaobaigu (BC_5F_3, 56.6%; BC_5F_2,74.2%) higher as well. The distribution of the seed setting rate of two populations (200 individuals of 'Towada^6/ Kunmingxiaobaigu' BC_5P_2 and 154 individuals of 'Towada^7/Kun-mingxiaobaigu' BC_6F_2) showed that all of them not only have two-peak distribution, with seed setting rates ranging from 8.2% to 96.2%, 0.6%-89.9%, respectively, but also the segregation ratios of strong: weak cold tolerance, were tested by χ^2 analysis against the expected ratio for a single gene (1:1). Therefore, these NILs are controlled by the major gene for cold tolerance.

NILs for cold tolerance at booting stage from artificial mutagenesis: We have bred one pair of NILs for cold tolerance at booting stage using 51 materials of artificial mutagenesis with 300 Gy, and the irrigation with 16~18°C water from a cool-water spring at Aziying (2150 m amsl), and at Kunming (1916 m amsl) under natural condition. This pair of NIL are similar to parent in most characters except the seed setting rate under cold condition. The NILs have clear difference in seed setting rate in contrast with the parent, with the difference exceeding 80%, in variety '02428' (1.0%)and its NIL (M_5 83.3%). 347 plants of F_2 and F_3 population of 02428/02428M_6 showed non-normal distribution, the cold tolerance gene of 02428M_6 suggest a major gene [9].

Chromosomal location of cold gene of NILs at booting stage in japonica rice: A total of 154 BC_4F_4 plants (NILs) of 'Towada^5/Kunmingxiaobaigu' from the backcross were analyzed by using 150 microsatellite markers [12], which distributed relatively uniformly throughout the genome, a good coverage of all the 12 chromosomes. Only RM31 in chromosome 5 was detected to be linked to cold tolerance gene at booting stage but markers in other chromosomes (1-4, 6-12) were not linked to the cold tolerance gene. 12 SSR markers [12] of near RM31 were used to further screen polymorphism between Towada and NIL, and found that RM7452 was polymorphic. So two markers (RM31, RM7452) markers were linked with the cold gene at booting stage through ONE WAY ANOVA of software SPSS. A molecular linkage map on chromosome 5 was constructed by using software QTL mapmaker 1.01b and Qgene 3.06. Using data of NIL of 154 plants of F_2 population of Towada × (Towada^5/ Kunmingxiaobagu BC_4F_5) for cold tolerance, the gene at booting stage mapped on chromosome 5 was found to be located between RM31 and RM7452, with the genetic distance of 4.8 and 8.0 cM, respectively [14]. However, it was found to be in a different study only 1.0 cM between RM31 and RM7452 on mapping of 2240 new SSR markers for rice [13]. Therefore, this cold tolerance gene of NIL from Kunmingxiaobaigu has very high utilization value and can be effectively used for alleviating cold stress by involving it in breeding programmes.

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