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Full Length Research Paper

Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *saltol* QTL

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A major quantitative trait locus (QTL) for salt tolerance named *Saltol* was mapped on chromosome 1 using F8 recombinant inbred lines (RILs) of Pokkali/IR29 cross, which is responsible for low Na^+, high K^+ uptake and maintaining Na^+/K^+ homeostasis in the rice shoots. To test the usefulness of microsatellite (SSR) markers associated with *Saltol* QTL, a collection of 36 diverse rice genotypes were used. Phenotypic response of the genotypes to salt stress with EC=12 was assessed under controlled environmental conditions at seedling stage using a visual score of 1 to 9 scale. Thirty three polymorphic SSR markers located on chromosome 1 were also used to determine the impact of these markers associated with salt tolerance in rice. The results of phenotypic response of rice genotypes to salinity stress at the seedling stage indicated the varied genotypic responses. The genotypes were classified into five groups from highly tolerant (score 1) to highly sensitive (score 9). Number of alleles of the SSR markers ranged from 3 for RM10702 to 14 for RM8094. Polymorphic information content (PIC) value varied from 0.28 for RM8095 to 0.88 for RM8094 with an average of 0.73. The SSR marker, RM8094, was found to be superior for analysis of genetic diversity in this study. Cluster analysis of the rice genotypes based on SSR data divided the genotypes into three groups each of which having 12, 8 and 16 genotypes including highly salt-tolerant IRRI elite lines (cluster 1), salt tolerant and moderate tolerant genotypes as well as Pokkali and FL478 (cluster 2), sensitive and highly sensitive genotypes (cluster 3), respectively. The impact of chromosome 1 for tolerance to salinity at the seedling stage in rice was emphasized by the results. Thirty six rice genotypes divided into 18 different haplotypes based on *Saltol* QTL located on chromosome 1 using Pokkali cultivar as the reference. The haplotypes possessing RM8094 and RM10745 markers could discriminate the tolerant genotypes and hence could be useful for marker-assisted selection of *Saltol* QTL.

Key words: Rice, salinity, SSR markers, *Saltol*, chromosome 1, haplotype diversity.

INTRODUCTION

Rice is one of the world's most important staple crops. Although rice is considered as a sensitive crop to salinity, it is one of the most widely grown crops in coastal areas frequently inundated with saline sea water during high tidal period (Akbar et al., 1972; Maas and Hoffman, 1977; Mori and Kinoshita, 1987). In the present, salinity is the second most widespread soil problem in rice growing countries next to drought and considers as a serious constraint to increased rice production worldwide (Gregorio, 1997).

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There exists tremendous variation for salt tolerance within species in rice, providing opportunities to improve crop salt-stress tolerance through genetic means. Some attempts to develop salt-tolerant genotypes were based on highly tolerant traditional rice cultivars i.e. Pokkali and Nona-Bokra. (Akbar et al., 1985; Gregorio and Senadhira, 1993).

DNA based molecular markers have been used extensively to assess the genetic diversity in most crop species. Due to high efficiency, reproducibility, easy-to-use, co-dominance nature and high degree of polymorphism, microsatellite markers or simple sequence repeats (SSRs) are widely-used as molecular markers for finger-

printing germplasm to assess genetic diversity, pedigree analysis, evolutionary studies and genome mapping (Yang et al, 1994; McCouch et al, 1997, Garland et al, 1999). Rice microsatellites have been demonstrated to be polymorphic between (Akagi et al., 1997; Chen et al., 1997) and within rice populations (Olufowote et al., 1997). The unveiling of the rice genome draft sequence in public domain has given a vast choice of SSR markers (18,828 nos.) throughout the whole genome (IRGSP, 2005). Microsatellite markers have been used effectively to map QTLs associated with salt tolerance (Lang et al., 2000 and 2001; Singh et al., 2007). A major QTL located on chromosome 1 was identified for salt tolerance using F_8 recombinant inbred lines (RILs) of Pokkali/ IR29 cross (Gregorio et al., 1997). This QTL governed the Na^+/K^+ uptake ratio and accounted for 64.3 to 80.2% of the phenotypic variation in salt tolerance. This chromosome 1 segment was further saturated using RFLP and SSR markers using the RILs (Bonilla et al., 2002). The identified QTLs for Na^+, K^+ and Na^+/K^+ uptake ratio accounted for 39.2, 43.9 and 43.2% of the phenotypic variation. This segment of the chromosome 1 was further fine mapped by using near isogenic lines (NILs) of IR29 using Pokkali as the donor with microsatellite markers (Niones, 2004). It should be mentioned that Pokkali has been the most widely used salt tolerant parent by rice breeders. A newly developed line FL478 derived from a cross between IR29 and Pokkali was used as a novel source of salinity tolerance at seedling stage (Walia et al., 2005). Eight QTLs were found responsible for the variation in their K^+ and Na^+ content, among which SKC1 distinguished as a major QTL for the K^+ and Na^+ shoot content and was mapped on chromosome 1, using F_2 populations derived from a cross between Niponbare and Koshihikari cultivars (Ren et al., 2005).

The objectives of present study were to (i) evaluate the genetic diversity of rice genotypes based on SSR markers located on chromosome 1 as the most important source of salinity tolerance at the seedling stage, (ii) assess the most discriminating SSR markers of salinity tolerance haplotypes with reference to Pokkali which is known possesses salinity tolerance QTL on chromosome 1 (*Saltol*), and (iii) identify salinity tolerance rice genotypes with putatively novel salinity tolerance sources.

MATERIALS AND METHODS

Plant materials and growing conditions

A collection of 36 diverse rice genotypes, including IR29 (salt sensitive) and FL478 (salt tolerant) lines as controls, were used (Table 1). The genotypes having varied response to salinity stress ranging from landraces to improved lines obtained from IRRI's Plant Breeding, Genetics, and Biotechnology Division (Table 1). Rapid screening method for evaluation of responses of genotypes to salinity stress at seedling stage as described by Gregorio et al. (1997) was used in a salinized (EC= 12 dS/m) culture solution for 21 days under controlled conditions in the IRRI phytotron at 34°C/29°C day and night temperatures with relative humidity of 70%. The genotypes were grouped under 1, 3, 5, 7 and 9 score categories for salt tolerance on the basis of relative seedling shoot and root growth 21 days after salinity treatment (Gregorio et al., 1997)

Microsatellite analysis

Genomic DNA was extracted from young leaf tissue according to Delaporta et al. (1983). Thirty three polymorphic SSR markers located on chromosomes 1 were used (Table 2). SSR primers were obtained from Invitrogen Corp., Carlsbad, California, USA. The PCR reaction was performed in a 25 µl volume using a PTC-200 MJ thermocycler (MJ Research, Inc., Waltham, MA). The reaction mixture contained 1x PCR buffer, 1.5 mM of MgCl2, 1.0 µl dNTPs, 0.5 µM of each primer, 1 U of *Taq*-polymerase and 100ng of template DNA. After 5 min of denaturation at 94°C, 35 cycles were performed with 1 min at 94°C, 45 s at 55°C, 1 min at 72°C, and a final extension step of 7 min at 72°C. PCR amplified products of were separated in a 8% polyacrylamide gel at 100 V for 2.5 h in 1 x TBE buffer and stained with SYBR Safe (from Invitrogen Corp., Carlsbad, California, USA). DNA banding patterns were visualized using Alpha Imager (Alpha Innotech Corporation, San Leandro, CA).

Data analysis

For each of the defined loci, SSR allelic composition was determined for each genotype. The program Power Marker version 3.25 (Liu and Muse, 2005) was used to calculate allele frequencies, alleles per locus and observed heterozygosity for each locus. Polymorphism information content (PIC) values which indicating the ability to distinguish between genotypes for each primer combination, was calculated as expected heterozygosity for polymorphic bands with the following formula (Anderson et al., 1993) using Power Marker version 3.25 (Liu and Muse, 2005):

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

Where Pij is the frequency of the jth allele for marker i, and summation extends over n alleles. Cluster analysis was performed according to the unweighted pair group mean algorithm (UPGMA) with the Nei et al. (1983) similarity index using Power Marker version 3.25 (Liu and Muse, 2005).

Haplotype diversity was prepared according to McCartney et al. (2004) and Liu and Anderson (2003).

RESULTS AND DISCUSSION

The results of phenotypic response of rice genotypes to salinity stress at the seedling stage indicated the varied genotypic responses. The genotypes were classified into five groups from highly tolerant (score 1) to highly sensitive (score 9) where 14 (highly tolerant), 4 (tolerant), 7 (moderate tolerant), 5 (sensitive) and 6 (highly sensitive) (Table 1). Most of the tested IRRI elite lines were ranked as highly tolerant to salt stress at the seedling stage. Rice cultivars showed different reaction to salt tolerance viz Pokkali, Kala-rata were ranked as highly tolerant genotypes while Karuna and Baothai ranked as highly sensitive genotypes. On the other hand, Iranian

Table 1. Salinity stress reactions (EC = 12 dS/m) of rice genotypes at the seedling stage.

Entry No.	Elite Line No.	Designation	Score	Reaction to salinity *
1	SAL 187	IR65209-3b-6-3-1	1	HT
2	SAL 271	IR65858-4B-11-1-2	1	HT
3	SAL 345	IR69588-4R-P-11-3	1	HT
4	SAL 518	IR72046-B-R-7-3-1-2	1	HT
5	SAL 534	IR71832-3R-2-2-1	1	HT
6	SAL 543	IR71899-2-1-1	1	HT
7	SAL 546	IR71991-3R-2-6-1	1	HT
8	SAL 547	IR71995-3R-1-2-2	1	HT
9	SAL 669	IR74099-3R-3-3	1	HT
10	SAL 699	IR74105-3R-2-1	1	HT
11	SAL 729	IR70023-4B-R-12-3-1	1	HT
12	IRGC 19928	Cherivirrupo	3	T
13	IRGC 26913	Kala-rata 1-24	1	HT
14	IRGC 37015	Bhirpala	5	I
15	IRGC 72958	IR4630-22-2-5-1-3	5	I
16	IRGC 108921	Pokkali	1	HT
17	FL 478	IR66946-3R-178-1-1	1	HT
18	SI	IR64	3	T
19	SAL 164	IR65185-3B-8-3-2	5	I
20	SAL 411	IR72046-B-R-4-3-2-1-2B-1	3	T
21	SAL 503	IR72043-B-R-6-3-3-3	5	I
22	SAL 764	IR72046-B-R-8-3-1-3	5	I
23	SAL 775	IR75000-69-2-1	5	I
24	SI	IR29	9	HS
25	IRGC 73061	MOJANG KOR 080265	7	S
26	IRGC 61181	BaoThai	9	HS
27	IRTP 11448	CN499-160-13-6	9	HS
28	IRGC 26908	Karuna	9	HS
29	IRGC 64845	TCA4 IRTP NO. 10931	7	S
30	IRTP 17223	Kindang Patong	7	S
31	Iranian Variety	ShahPasand	3	T
32	Iranian Variety	Anbarbu	7	S
33	Iranian Variety	Tarom	9	HS
34	Iranian Variety	Sadri	5	I
35	Iranian Variety	Hassany	7	S
36	Iranian Variety	Gharib	9	HS

* HT = highly tolerance, T = tolerance, I = intermediate, S = sensitive and HS = highly sensitive.

cultivars were sensitive to salt stress with the exception of ‘ShahPasand’ (tolerant) and ‘Sadri’ (moderate tolerant) cultivars.

All the used SSR markers amplified polymorphic bands using 36 rice genotypes (Table 2). The lowest amplicon size belonged to RM490 (73 bp) and the highest amplicon size belonged to RM10772 (386 bp). The number of microsatellite alleles of used markers ranged from 3 to 14 of which RM8094, RM8115 and RM10772 markers (14, 13, and 12 respectively) produced the highest numbers of alleles while RM10702 and RM113 produced the lowest (Table 2). Polymorphic information content (PIC) value varied from 0.28 to 0.88 with an average of 0.73, the highest value belong to RM8094, RM10772 and RM10794 (PIC > 0.86) while RM8095 showed the lowest PIC value (PIC = 0.28). The SSR marker, RM8094, was

Table 2. Number of alleles and polymorphism information content (PIC) value of SSR markers for 36 rice genotypes.

Marker	Frequency of major allele	No. of allele	Locus heterozygosity	PIC value	Amplicon size range (bp)
RM9	0.36	7	0.79	0.76	151-194
RM14	0.31	11	0.83	0.81	167-281
RM23	0.36	5	0.74	0.69	155-161
RM81	0.36	7	0.76	0.73	91-109
RM113	0.53	3	0.60	0.53	155-165
RM140	0.58	5	0.60	0.56	248-264
RM142	0.56	6	0.64	0.61	129-135
RM220	0.58	7	0.62	0.59	99-127
RM243	0.17	11	0.88	0.87	96-118
RM323	0.61	4	0.56	0.51	226-251
RM329	0.53	4	0.64	0.59	97-112
RM449	0.25	8	0.83	0.81	107-143
RM490	0.25	8	0.84	0.83	73-94
RM493	0.42	9	0.78	0.76	193-253
RM513	0.31	7	0.79	0.76	269-290
RM562	0.22	8	0.84	0.82	232-280
RM594	0.22	9	0.85	0.83	298-328
RM595	0.28	7	0.82	0.80	192-225
RM1287	0.31	11	0.84	0.82	147-192
RM3412	0.36	11	0.82	0.81	225-260
RM5365	0.25	8	0.83	0.81	177-203
RM6681	0.22	10	0.85	0.83	181-305
RM8094	0.22	14	0.88	0.89	166-220
RM8095	0.83	4	0.30	0.28	187-212
RM8115	0.33	13	0.83	0.82	110-284
RM10702	0.69	3	0.46	0.41	273-276
RM10745	0.47	6	0.71	0.68	182-201
RM10764	0.25	8	0.81	0.78	131-171
RM10772	0.17	12	0.90	0.88	321-386
RM10825	0.19	8	0.85	0.83	76-92
RM10839	0.28	10	0.82	0.80	197-269
RM10916	0.22	10	0.85	0.84	200-269
RM10974	0.14	11	0.89	0.88	175-220

found to be superior for analysis of genetic diversity in this study. The polymorphic banding pattern of RM8094 marker in 36 rice genotypes is presented in Figure 1.

Cluster analysis of the rice genotypes based on SSR data divided the genotypes into three groups (Figure 2). First cluster comprised 12 genotypes of highly salt-tolerant improved IRRI elite lines, 6 salts tolerant and moderately tolerant genotypes as well as Pokkali and FL478 cultivars belonging to cluster 2, and 16 genotypes of sensitive and highly sensitive genotypes as well as all Iranian genotypes belonging to cluster 3. The impact of chromosome 1 for tolerance to salinity at the seedling stage in rice was emphasized by the results (Figure 2). A clear relationship between morphological and molecular data was evident and a significant difference was found for scoring data among three cluster groups (F= 48.6 **).

Among 33 SSR markers on chromosome 1, eight tightly linked SSR markers to the *Saltol* positioning at 10.8 to 12.28 Mb (www.gramine.org) were used for haplotyping (Figure 3). Eighteen haplotypes were identified among the 36 rice genotypes (Table 3). Ten genotypes allocated to 10 single haplotypes. None of the 35 genotypes produced similar haplotype as Pokkali. In the other words, none of the used genotypes possess all of the microsatellite markers belonging to Pokkali. Twenty-nine genotypes had different combinations of some Pokkali's alleles, while six genotypes did not have any common marker alleles with the Pokkali haplotype (haplotype number 18).

From comparison of the haplotypes with Pokkali haplo-

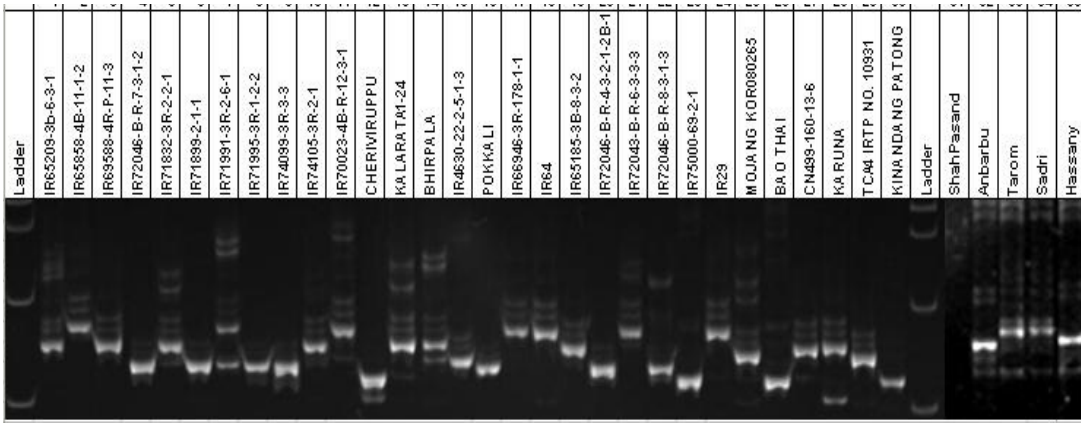

Figure 1. DNA bands amplified from leaves of 36 rice genotypes using microsatellite RM8094 marker and electrophoresed in a 8% polyacrylamide gel. Ladder = 100 bp ladder.

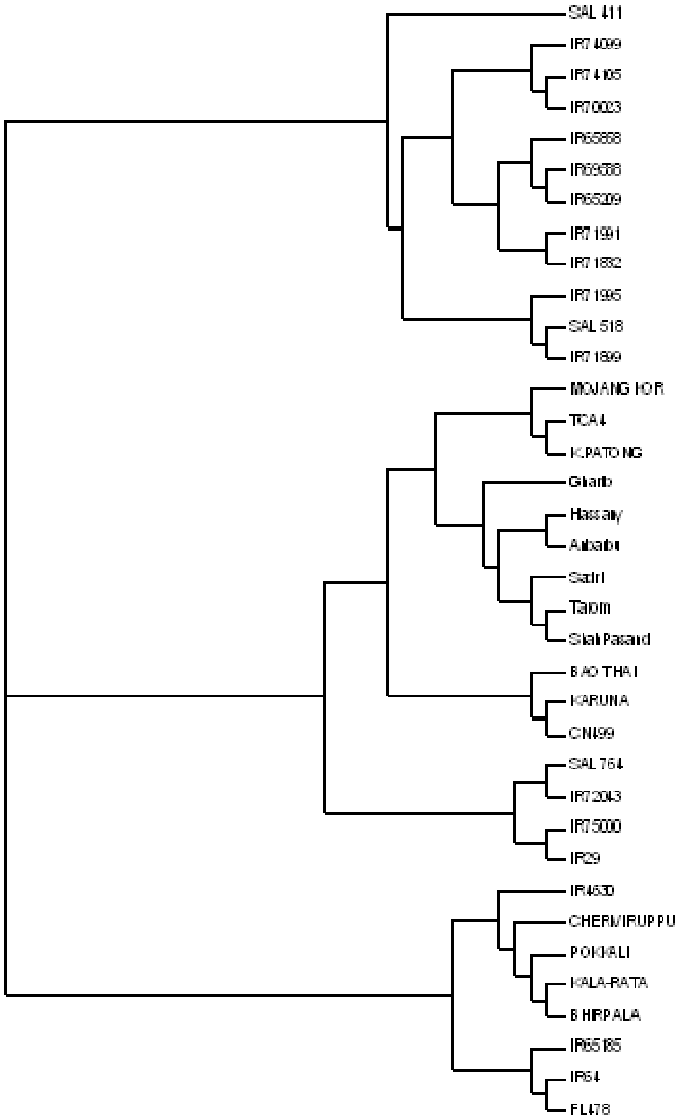

Figure 2. Dendrogram of 36 rice genotypes based on 33 polymorphic SSR markers on chromosome 1 according to the un-weighted pair group mean algorithm (UPGMA) with the Nei's similarity index.

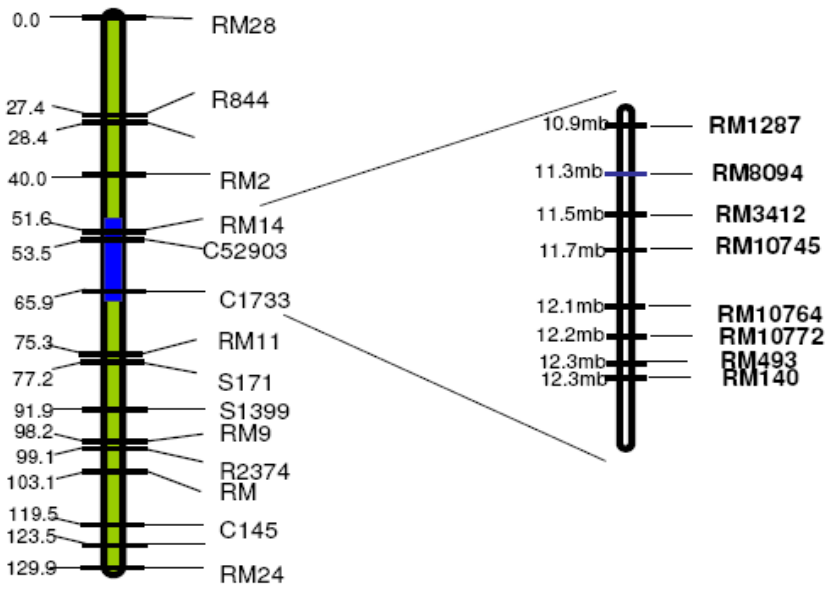

Figure 3. *Saltol* segment on chromosome 1 of rice (Bonila et al., 2002; Niones, 2004) with new arrangement of used SSR markers according to IRGSP (2005).

Table 3. Eighteen rice haplotypes produced by SSR markers located on *Saltol* QTL region on chromosome 1 with reference to Pokkali*.

RM1287	■		■					■										
RM8094	■	■							■	■	■			■				
RM3412	■	■		■					■			■			■	■	■	
RM10745	■	■			■					■		■	■	■		■		
RM10764	■										■							
RM10772	■					■							■		■		■	
RM493	■	■												■				
RM140	■						■	■							■	■	■	
Haplotype No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

*1 = POKKALI; 2 = FL478; 3 = ShahPasand, Tarom; 4 = IR75000, MOJANGkOR, BAOTHAI; 5 = IR64, KALA-RATA , IR71991, IR72043; 6 = IR29; 7 = Hassany; 8 = SAL411, Sadri; 9 = Cherivirrupo; 10 = IR65858, IR69588, IR74105, IR70023; 11 = IR74099; 12 = IR71899, IR65209, IR4630, K.PATONG; 13 = BHIRPALA; 14 = IR71832; 15 = SAL518; 16 = SAL764; 17 = IR71995; 18 = TCA4 , IR65185, Anbarbu, CN499, KARUNA, Gharib

type, it can be assumed that the rice genotypes possessing the Pokkali band type for locus RM8094 marker, they were either highly tolerant or tolerant to salinity stress at the seedling stage (Table 3). IR70023, IR65858, IR69588, IR74105, IR71832 and IR74099, Cherivirrupo and IR66946-3R-178-1-1 which is known as FL478 had the Pokkali marker allele for the RM8094 marker and all were found to be either highly tolerant or tolerant to Salinity stress (Table 3). Therefore, this marker appears to have a strong and positively association with seedling salt tolerance in rice. Some of salt-tolerant genotypes had the pokkali marker allele for RM10745. The genotypes that had the same marker allele as Pokkali for the RM3412 and RM1287 markers showed different reaction to salinity while Pokkali's markers RM8094 and RM10745 consistently discriminated the salt tolerant genotypes (Table 3).

Rice haplotype which did not contain any common marker alleles with Pokkali haplotype responded either sensitive or highly sensitive to salinity stress (Table 3). IR66946-3R-178-1-1 (also known as FL478) line derived from IR29/Pokkali cross and used as tolerant control in this study had a highly tolerant response and was the mostly related genotype to Pokkali considering their common RM8094, RM3412, RM493 and RM10745 markers.

Based on the previous report it was found that five SSR markers (RM1287, RM8094, RM3412, RM493 and

RM140) and two EST markers (CP3970 and CP6224) were linked to *Saltol* QTL on chromosome 1 (Niones, 2004). It is also observed that that *Saltol* locus is probably located within a region consisting of RM8094, RM3412 and RM493 markers (Islam, M. R. personal communication). The results of present study further verified that RM8094 and RM10745 are useful for marker-assisted selection of *Saltol* QTL.

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