Title: ENHANCEMENT OF SALINITY TOLERANCE IN RICE

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2000 to December 31, 2000

Title: ENHANCEMENT OF OSMOTIC (SALINITY) STRESS TOLERANCE IN RICE
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LEVEL OF 1998 FUNDING: $7,000
REPORT BY OBJECTIVE AND CONCISE SUMMARY

(Objective 1) Identification of PCR-based markers closely linked to the *Saltol* locus which controls K^+/Na^+ selectivity and salt tolerance in the *indica* variety Pokkali.

Indica-type rice variety Pokkali was identified at IRRI as the top salt tolerant variety in their salt stress tolerance screening program. In most cereals, including rice, K^+/Na^+ selectivity correlates with salt stress tolerance. Consistently with this observation, Pokkali shows a high level of K^+/Na^+ selectivity. Pokkali was crossed with salt sensitive IR29 and a total of 78 putative recombinant inbred lines (RILs) were developed. These were used by Glen Gregorio and Philbert Bonilla (PhD thesis, IRRI) to map K^+/Na^+ selectivity with amplified fragment length polymorphism (AFLP) markers. A quantitative trait locus (QTL) affecting K^+/Na^+ selectivity was associated with a region on chromosome 1. Two AFLP markers (P3/M9-8 and P1/M9-3) linked to this putative K^+/Na^+ selectivity locus (designated *Saltol*) were discovered.

To map more precisely the *Saltol* locus relative to molecular markers and to identify PCR-based markers flanking the locus, work on the construction of a more

detailed map based on these RILs was initiated in year 2000. A total of 39 RFLP markers mapped on chromosome 1 by the Japanese Rice Genome Project and Cornell University were screened. Polymorphism between Pokkali and IR29 was detected for 22 markers and these were mapped. Hybridization with these RFLP markers revealed that of the initial 78 RILs, 24 lines were heterozygous due to outcrossing or other reasons and were eliminated from the mapping population. This improved the precision of the map and the position of the *Saltol* locus.

PCR-based markers, such as microsatellites, are far more useful in breeding programs than AFLP or RFLP markers. To isolate microsatellite markers flanking the *Saltol* locus, Pokkali and IR29 were screened for polymorphism with 24 pairs of microsatellite primers. Seven microsatellite loci (designated as RM in Fig. 1) were polymorphic and were mapped in the *Saltol* region on chromosome 1. Microsatellite markers RM140 and RM 113 flank the locus (Fig. 1).

Because of the small size of the Pokkali x IR29 RIL mapping population, the estimates of the interval lengths of the Pokkali x IR29 map are burdened by large variance. To assess the reliability of the Pokkali x IR29 RIL map, the map is compared with the map based on the Niponbare x Kasalath F_2 population (Fig. 1). Both maps show the same order of markers, indicating that the order of markers is correct on the Pokkali x IR29 map. However, RFLP markers C1905 and C1733S delineating interval encompassing the *Saltol* locus are 32.7 cM appart on the Pokkali x IR29 RIL map but only 4.7 cM on the F_2 Niponbare x Kasalath map. The estimate of 4.7 cM for the interval including the *Saltol* locus should be considered more realistic than the estimate based on the Pokkali x IR29 RIL population.

The microsatellite markers RM140 and RM113 flanking the locus must also be more tightly linked to *Saltol* than is apparent on the Pokkali x IR29 RIL map (Fig. 1). Using markers shared by both maps (Fig.1), the maximum distance between the two microsatellite markers is 12.6 cM. This linkage is adequate for tagging the *Saltol* locus in breeding programs and saturation mapping for cloning of the locus.

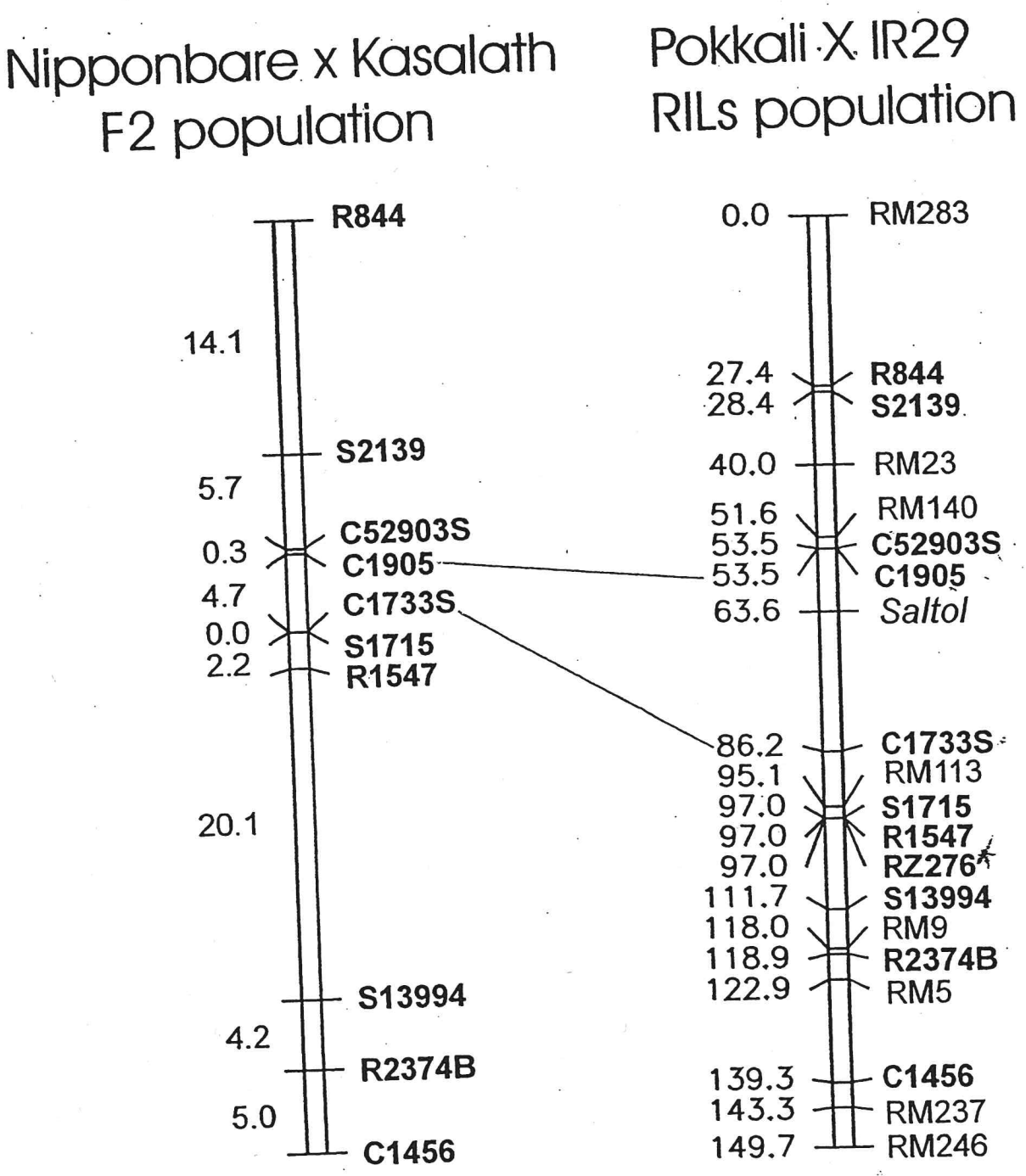

Figure 1. The position of the *Saltol* locus on the map of rice chromosome 1 (54 F_8 RILs from the cross Pokkali x IR29) based on AFLP markers (P/M), microsatellite markers (RM) and RFLP markers (the rest). Japanese Rice Genome Project map based on F_2 Niponbare x Kasalath population is shown for comparison of marker order and lengths of intervals. Note that the two maps are co-linear. The *Saltol* locus is flanked by RFLP markers C1905 and C17332 delineating an interval of 32.7 cM. The same interval is only 4.7 cM on the Niponbare x Kasalath map.

(Objective 2) Backcrossing of the *Saltol* locus from Pokkali to M-202 using molecular markers (in collaboration with P. Bonilla, PhilRice).

Two accessions of Pokkali were received from the Small Grains Germplasm Collection in Aberdeen, Idaho. Both were tested for K^+/Na^+ selectivity and both turned out to have selectivity similar to that of M202 (medium high). We conclude that Pokkali, being a landrace, is polymorphic for K^+/Na^+ selectivity and *Saltol* and that the US accessions may not have the *Saltol* gene. We therefore made arrangements with Glen Gregorio in IRRI to cross Pokkali which was the parent of the Pokkali x IR29 mapping population with M202. He made the cross and is now backrossing the F_1 to M202. The BC_1 generation will be shipped to us in year 2001 and will be grown under quarantine conditions. The population will be backcrossed to M202.

(Objective 3) Completion of screening of exotic rice accessions for K^+/Na^+ selectivity and crossing three best lines with M202

Work on this objective was limited by inadequate seed supplies left after the previous screening cycles. We therefore increased seeds of the critical materials during the 2000 season.

(Objective 4) Characterization of a rice contig (a sequence of clones representing a contiguous DNA sequence in a chromosome) spanning the *Kna1* (K^+/Na^+ selectivity controlling locus) region in wheat.

The wheat *Kna1* locus resides in the distal region of chromosome 4D. Rice BAC clones hybridizing with wheat genomic probe WG199, which is completely linked to *Kna1*, were isolated and mapped. The BAC contig identified by this hybridization was found to map to the centromeric region of chromosome 8. Further characterization revealed that this contig is paralogous to the *Xwg199* locus on chromosome 4D. The orthologous region has not been found in rice.

An additional snag immerged during characterization of 4B/4D recombinants. It turned out that the chromosome region containing the *Kna1* locus on wheat chromosome 4D differs from wheat chromosome 4B by a small paracentric inversion including the *Kna1* locus. This precludes construction of a saturation map and positional cloning of the locus on the basis of recombination between wheat chromosomes 4B and 4D. We therefore initiated search for polymorphism at the *Kna1* locus on chromosome 4D in *T. aestivum*. Finding such allele would make it possible to employ a saturation mapping strategy based on 4D x 4D recombination rather than 4D x 4B recombination. So far, we have not found any wheat genotype with a recessive *kna1* allele.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS

A major locus controlling salt tolerance in rice, *Saltol,* was mapped relative to molecular markers on the long arm of rice chromosome 1. The locus was placed into the 4.7 cM C1905 and C1733S interval. To facilitate rapid selection of *Saltol* in segregating populations, the locus was mapped relative to microsatellite markers. Microsatellites RM140 and RM113, less than 12.6 cM apart, flank the locus. In conclusion, we have accomplished our primary goals (1) to identify a source of salt tolerance for breeding for salt tolerance in California and (2) to develop breeder-friendly molecular markers for salt tolerance breeding in rice. Introgression of *Saltol* into California rice germplasm was initiated in collaboration with Glen Gregorio (IRRI) and work on this objective is being continued.