TARGETED ASSOCIATION ANALYSIS FOR TOLERANCE TO SALINITY WITHIN THE EUROPEAN CORE COLLECTION OF TEMPERATE JAPONICA RICE

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In INTRODUCTION

In Europe, rice is grown mainly in deltaic areas with salinity problems. In the framework of successive EU-funded projects called RES-GEN and EUROGEN, rice breeding programs from France, Greece, Italy, Portugal and Spain have established a European Rice Genetic Resources Collection (ERGRC) of some 450 accessions, mainly temperate japonica, extensively characterised for agronomic traits and maintained by CIRAD. The aim of this work was to identify, within this collection, a set of best performing genes and alleles for tolerance to salinity, as well as the associated donors and molecular markers for use in breeding programs.

MATERIAL AND METHODS

Establishment of an association analysis panel of 200 accessions: The 450 accessions of ERGRC were genotyped using 26 SSRs markers following the protocol of Roy et al. (1996) applied with the automated infrared fluorescence technology of LICOR IR2 sequencers in the genotyping and robotics platform of Cirad. The number of subgroups within the ERGRC was assessed using Structure software (http://pritch.bsd.uchicago.edu/structure.html) and Evanno’s index (Evanno et al 2005). A subset of 200 accessions for the association panel were selected using DarWin software (http://darwin.cirad.fr/darwin/Home.php).

Targeted SNP genotyping: A database of rice QTLs and candidate genes for salinity tolerance was developed (http://tropgenedb.cirad.fr/html/rice_QTL.html) and both QTLs and genes were mapped on the rice physical map. Using this map, a set of 240 target candidate genes was chosen based on their co-localization with QTLs and on the supposed function of the gene family. Using OryzaSNP database (http://www.oryzasnp.org/), 1029 SNPs responding to Illumina genotyping technology were identified, of which only 213 were polymorphic among the 7 japonica accessions of the OryzaSNP database. These SNPs covered only 60 salinity tolerance candidate genes. Finally, the association panel, was genotyped with 124 SNPs located in 47 candidate genes.

Targeted association analysis: Using Tassel software (http://www.maizegenetics.net/tassel/), association analysis was performed based on the linear model using the percentages of admixture estimated in the analysis of population structure as cofactors in the analyses.

RESULTS AND DISCUSSION

Organization of the genetic diversity within the ERGRC: The most likely number of subgroup was 3 (Figure 2). Accessions that could not be assigned to one of the 3 groups for more than 80% of their genotype were considered as admixed. Beside temperate japonica (Gg1 and Gg3), the ERGRC included a significant number of indica (Gg2) and some basmati-type accessions. Within the japonica group, accession with American background formed a distinct branch (Gg1). A sub-sample of 200 accessions maximizing simultaneously allele number and allelic associations was then chosen, mainly among the temperate japonica group, to form the association analysis panel.

Candidate genes associated with tolerance to salinity: Only 80 SNPs representing 41 candidate genes were polymorphic within the association panel. No polymorphic SNPs were found for major salt tolerance QTLs. Among the 80 SNPs, 37 representing 25 candidate genes were significantly associated with one or more phenotypic traits (Table 1). Five candidate genes (red color in Table 1) were associated with growth and development parameters specifically under salinity treatment while 7 other (green color in Table 1) were specific of control treatment. The candidate gene associated with Na+ concentration, located on chromosome 5, was also associated with tillering and leaf emission and biomass production under stress.

The results of this targeted association mapping are encouraging but the major salinity tolerance QTLs and candidate genes could not represented in the tested SNP set. The set of markers is presently being extended to be able to test these QTLs/genes as well.

Salinity tolerance of 200 accessions was evaluated through comparison of stressed and control plants for growth parameters, biomass production - Plant Height (PH), Number of tiller (TN), Number of leaf (LN), Shoot dry weight (SDW). Root dry weight (RDW) - and concentration of Na+ and K+ ions in the shoots at 38 days after sowing (Figure 1). For each measured variable X, an index of response (r) to salinity was computed: X_r = (X (c) - (X (s)/100) / X_c, were X_c and X_s are the X variable under optimal growth and under salinity stress, respectively. The experimental design, a split plot with 2 factors, included 4 replications, and seven check varieties. The tolerant checks were Pokkali and Nonabokra; the susceptible checks were IR 29 and Aichade; and 3 well known varieties, Nippobare, Fidji and Giano, were also included.

Diversity for salinity tolerance: Response to salinity stress was an overall reduction of growth and biomass. Salinity also affected significantly Na+ (mean increase of 600%) and K+ (mean decrease of 39%) resulting in high Na+ / K+ ratio. Genotype by salinity treatment interaction was highly significant for all measured and computed variables confirming the existence of genetic diversity for response to salinity among the 200 accessions of the association panel. Ascendant hierarchical classification of response to salinity leaded to identification of 3 phenotypic groups (Figure 3). Gp1 was significantly different of Gp2 and Gp3 for response variable. Gp2 was significantly different of G3 for all response variables but Na+ / K+ ratio.

Table 1: Association between 25 candidate genes and plant growth and development parameters under control (c) and salinity stress (s).

Figure 1: Salinity stress during the vegetative stage in hydroponic conditions.

Figure 3: Ascendant hierarchical classification of the 200 accessions of the association panel based on growth and development parameters to salinity response.