Study of cold tolerance at vegetative and reproductive stage in Chilean temperate rice

GH Donoso [A*], MO Paredes[A], VL Becerra[A].

[A] Center of Food Biotechnology, Institute of Agricultural Research, INIA Quilamapu, Chillán City, Chile.
Keywords: seedling, microsporogenesis, Multivariate analysis and BLUP.

Introduction

Chile is the southernmost country where rice (*Oryza sativa* L.) is cultivated. In this country, the temperatures are under the optimal needed for rice cultivation, with a minimal temperature between 5ºC to 10ºC. This temperature induces a cold stress in rice plant and can cause a decrease in plant density, spikelet fertility and grain yield. Therefore, the study of cold tolerant genotypes at vegetative and reproductive stage it is very important to increase the effectiveness of Rice Breeding Program of Chile. By this means, the objective of this work was to study cold tolerance of Chilean temperate rice at vegetative and reproductive stage.

Material and Methods

At vegetative stage, Seeds from 90 genotypes from Breeding Program Rice from INIA Quilamapu, Chile, were evaluated. For this, seeds were germinated on absorbent paper with a solution of the fungicide Benlate (2 ppm) at 28° C in the dark for 72 h. After germination, seedlings were transplanted into plastic pots of 500 mL with clay soil (Vertisol). Plants were grown in a greenhouse with a photoperiod of 14 h of light and 10 h of dark, at 28 °C day and 22 °C night. The plants were illuminated with artificial light using red and blue LEDs light, and metal halide lamps, with a radiation of 300 mmol photons m^{-2} s^{-1}. Seedlings with 3 to 4 expanded leaves were treated at 5° C in a cold chamber in darkness for 72 h. Finally, three plants per pot were sampled and frozen in liquid nitrogen and stored at 80° C until analysis. Chlorophyll concentration, atLEAF values (Chorophyll meter), carotenoids concentration, proline concentration, and visual damage were measured ten days after cold treatment. The classification of genotypes was performed using BLUP predictors calculated for each trait evaluated, and principal components analysis. At reproductive stage, 150 genotypes were grown in a 10 L plastic pot with rice soil (Vertisol), under mesh with 36 % of light reduction, until microsporogenesis stage. This stage was determined using auricular distance, previous verification based in microscopy analysis. At this stage, plants were treated with low temperature (5 °C) for 24 h, under dark with Ambar-INIA and Susan as cold tolerant genotypes. After stress, plants were returned to field conditions for recovery, and ratio of grain weight per panicle between control and treated plants, was compared. The genotypes evaluated at vegetative and reproductive stage, were classified according to calculated the best linear unbiased prediction values (BLUP) for each traits. For evaluation at vegetative a multivariate analysis based in Principal Component was made using InfoStat (Di Rienzo *et al*., 2011). Broad-sense heritability for all traits was calculated using equation described by Doligez et al. (2013): $H^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_\varepsilon)$, where: H^2 = Broad sense heritability, σ^2_G = Genotypic variance, σ^2_ε = Residual Variance.

Results

At vegetative stage the check genotype, Susan, showed cold tolerance values in four rankings (Visual damage, atLEAF values, Chlorophyll concentration and carotenoids) and Oryzica 1 showed cold susceptible values in all rankings (Figure 1).

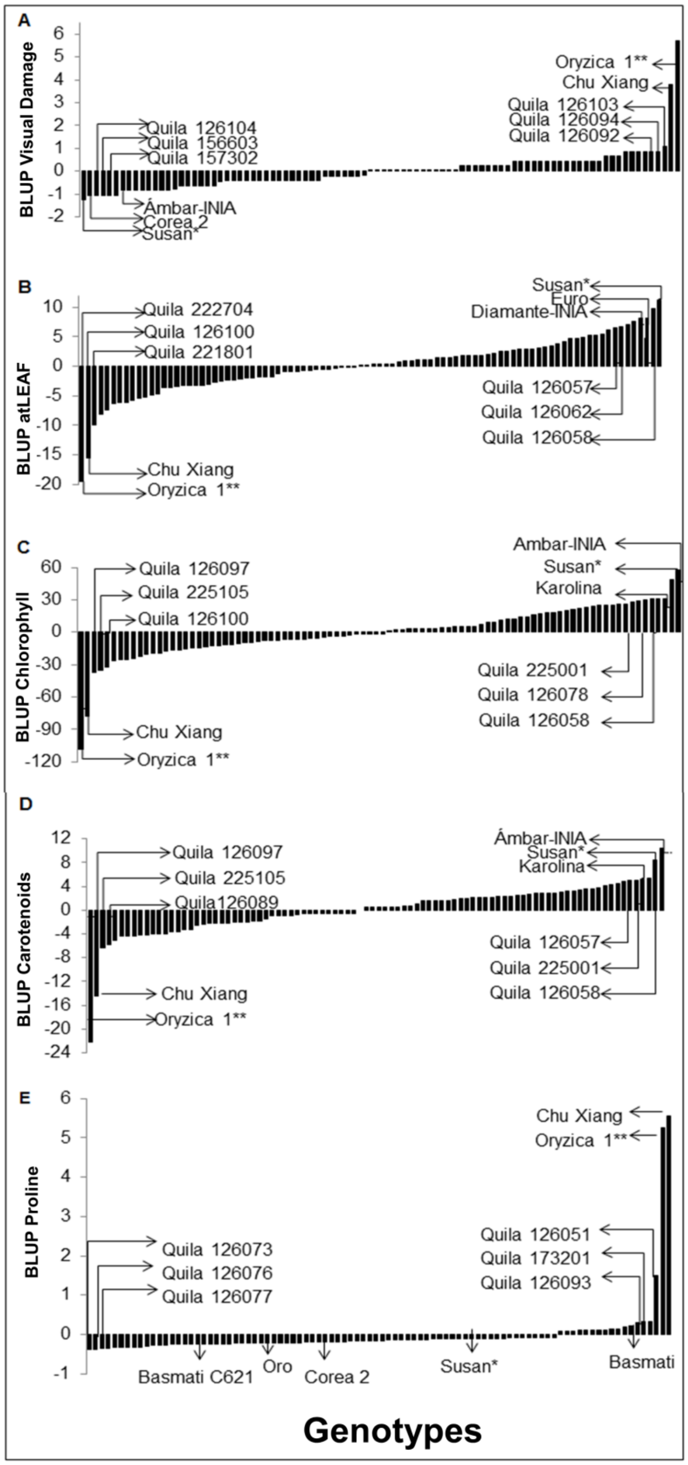

Figure 1. Ranking based in Blups for five traits. A, Visual damage; B, atLEAF value; C, Chlorophyll; D, Carotenoids; E, Proline and F Principal Component Analysis using all traits evaluated.

Quila 126058, Quila 126057 and Quila 126062, were considered as cold tolerant, while Quila 126097, Quila 126103 and Quila 126100, were considered susceptible genotypes (Figure 2). Low heritability was observed for chlorophyll and carotenoid content (table 1). Furthermore, we find that proline content did not allow finding differences among genotypes with intermediate cold tolerance.

At reproductive stage, the 96 % of the genotypes were negatively affected for the low temperatures (Figure 2). Six genotypes presented a high ratio of total grain weight per panicle (Quila 159005, Quila 242114, Quila 233008, RQuila 418, Quila 249304 and Quila 230602.) and 13 genotypes showed a very low ratio.

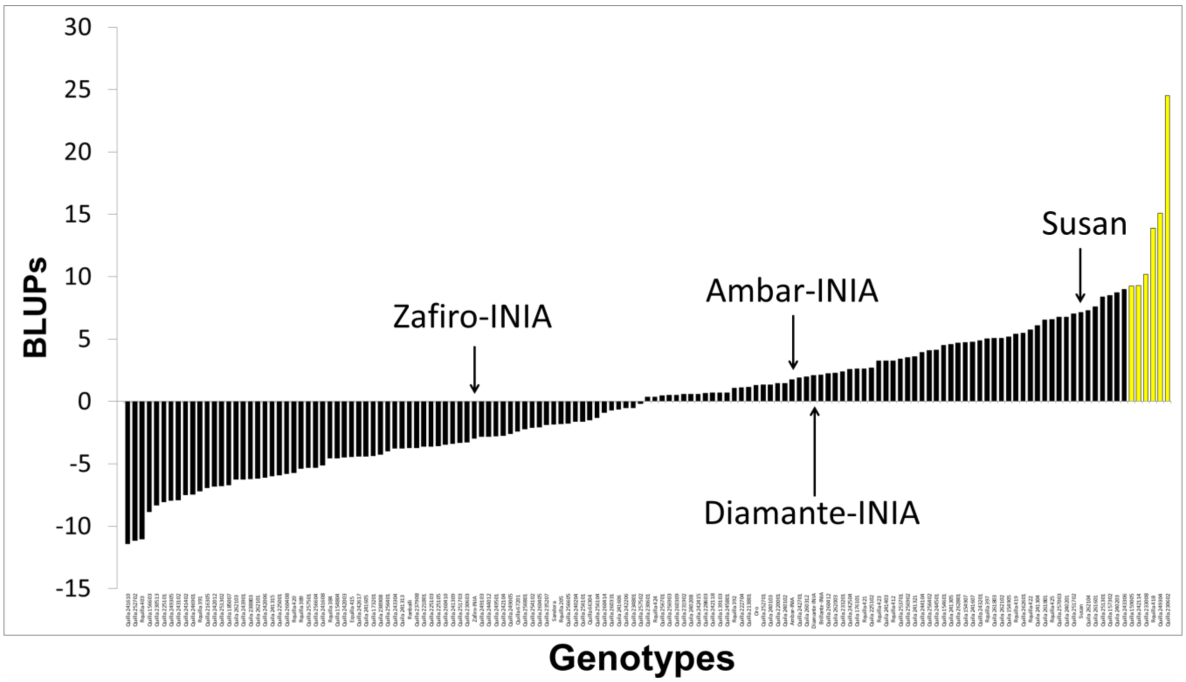

Figure 2. Ranking for cold tolerance of 150 rice genotypes at reproductive stage (microsporogenesis). Yellow bar represent the cold tolerant genotypes.

Conclusion

In conclusion for vegetative stage evaluation, the best trait related with cold tolerance was atLEAF value, with good heritability and normal distribution. This is a nondestructive, easy and objective methodology. Therefore, chlorophyll content is the most suitable parameter to evaluate cold tolerance in rice at seedling stage. In conclusion for reproductive stage evaluation, auricular distance was a good predictor for pollen grain development, auricular distance between -5 cm to 0 allow us to find tetrad stage in the most of the genotypes and low number of experimental lines were cold tolerant at reproductive stage.

Grant: FONDEF D10E1183, Tucapel SA, Carozzi and BASF.