RESEARCH

Cold tolerance evaluation in Chilean rice genotypes at the germination stage

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Low temperature is the most important abiotic stress affecting rice (*Oryza sativa* L.) yield in Chile. Rice in Chile is usually planted when the minimum air temperatures are below 12 °C. This temperature is lower than the optimum needed for normal rice germination. Therefore, the aim of this study was to evaluate cold tolerance in 20 experimental lines from the Rice Breeding Program of the Instituto de Investigaciones Agropecuarias (INIA), Chile, at the germination stage. Coleoptile length reduction (CRED), coleoptile length after cold treatment (CLEN), coleoptile length recovery (CREC), and coleoptile regrowth (CREG) were evaluated at 13 °C for 4 d using 'Diamante-INIA' as the cold-tolerant control. To find genotypes with cold tolerance (low CRED value and high CLEN, CREC, and CREG values), genotypes were ranked, a biplot of principal components, and cluster analysis were performed. No differences were found among genotypes in the ranking based on CREC value so this trait was not considered. Analysis showed that only three experimental lines had cold tolerance similar to that of 'Diamante-INIA'; all other experimental lines exhibited intermediate to low cold tolerance. These results showed low cold tolerance of some Chilean genotypes at the germination stage, thus confirming the need to evaluate the rest of the germplasm from the Rice Breeding Program.

Key words: Low temperatures, coleoptile length, experimental lines.

INTRODUCTION

Low temperatures are responsible for high yield loss in rice (*Oryza sativa* L.) production in temperate areas such as Australia, Japan, China (Yoshida, 1981), and Chile (Alvarado and Hernaiz, 2007). Chile is one of the coldest regions where rice is cultivated. The rice area in Chile has a Mediterranean climate and is located between the Libertador General Bernardo O'Higgins Region and the Biobío Region (34° to 36° S). Rice in Chile is planted with pre-germinated seeds in a flooding regime (Alvarado and Hernaiz, 2007) during early spring with minimum air temperatures below 12 °C. These temperatures are below the optimum temperature for rice germination (Yoshida et al., 1996).

Exposure to cold temperatures affects all the phenological stages of rice. Germination under low temperatures can cause slow growth and reduce seedling vigor (Ali et al., 2006). Cold stress at the seedling stage can cause a low number of seedlings, reduce tillering (Shimono et al., 2002), increase plant mortality (Mackill and Lei, 1997; Andaya and Mackill, 2003; Fujino et al., 2004; Baruah et al., 2009), and also induce non-uniform crop maturity (Shimono et al., 2004). Low temperatures at the rice vegetative stage increases the growth period (Alvarado and Hernaiz, 2007). On the other hand, cold stress at the reproductive stage produces panicle sterility and lower grain production and yield (Shimono et al., 2007). In all cases, the level of damage associated with cold stress will depend on the development stage and intensity of the cold event (Jacobs and Pearson, 1994).

Screening for cold tolerance in rice has proven to be more difficult than expected because the correlation between cold tolerance and the germination and reproductive stages was low or zero. This is logical since both stages involve completely different physiological processes. Furthermore, traditional breeding programs have had limited success in improving cold tolerance, mostly due to the multigenic nature of the trait, unpredictability of the weather, and interaction between cold response and environmental factors such as light and soil nutrient availability (Mittler, 2006). To address this problem, many methodologies have been developed to evaluate cold tolerance in rice at the germination stage: germination index at 13 °C for 28 d, coleoptile growth at 13 °C for 3 d, and coleoptile regrowth at 28 °C for 4 d (Cruz and Milach, 2004); imbibition rate, germination rate, germination index, root length, shoot length, and seed vigor at 14 °C for 23 d (Wang et al., 2009); germination coefficient at 10, 13, and 15 °C (Baruah et al., 2009); germination rate, coleoptile length, and radicle length at 13 and 17 °C for 28 d (Sharifi and Aminpanah, 2010). Cold tolerance evaluated by Cruz and Milach (2004) is a good approximation to assess cold tolerance

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Received: 29 December 2012.
Accepted: 28 December 2013.

at the germination stage because they considered the coleoptile reduction and coleoptile regrowth after cold. This experiment allowed identifying parents and crosses with high cold tolerance.

Only one study has been conducted in Chile to evaluate cold tolerance at the germination stage (Castillo and Alvarado, 2002). One hundred and eighty-four accessions were evaluated after cold treatment at 13 °C for 5 d with 'Diamante-INIA' as a cold-tolerant genotype. The Rice Breeding Program of INIA Chile has developed more than 3500 potentially cold-tolerant experimental lines, whose tolerance at the germination stage has not been investigated yet. Therefore, the evaluation of genotypes to detect high cold tolerance at the germination stage could be very important to improve rice varieties in Chile. The present study proposes evaluating cold tolerance at the germination stage for experimental lines from the Rice breeding Program of INIA Chile based on Cruz and Milach's (2004) methodology and multivariate analysis by Bosetti et al. (2012).

MATERIALS AND METHODS

Plant material, germination, and cold treatment

The genotypes included in this study were: four cultivars from Chile, 20 experimental lines from Chile, two cultivars from Spain (*japonica*), one cultivar from Hungary (*japonica*), one cultivar from India (*indica*), and one cultivar from China (*indica*) (Table 1). 'Diamante-INIA' was used as a cold-tolerant genotype and 'Sugandh-2' was used as a cold-susceptible genotype.

Cold tolerance at the rice germination stage was evaluated according to a protocol by Cruz and Milach (2004), which was modified. Germination occurred in magenta vessels with 100 g of vermiculite moistened with 100 mL of distilled water. Seeds from each genotype were germinated under cold and control conditions. The cold condition included 30 seeds from each genotype, which were germinated in the dark at 28 °C for 3 d in a growth chamber (BK-600, Heraeus Instruments, Hanau, Germany) and then the chamber temperature was set at 13 °C for 4 d. Finally, the chamber temperature was set at 28 °C for another 3 d. The control condition included 30 seeds from each genotype which were germinated at 28 °C for 10 d. The coleoptile length of each genotype in the two conditions was measured at 7 d and 10 d after sowing. The reduction of coleoptile length after cold treatment (CRED), reduction of coleoptile length after regrowth (CREC), and coleoptile regrowth (CREG) were analyzed and calculated with the formulae:

CRED = (Coleoptile length of control 7 d after sowing) - (Coleoptile length after cold treatment)

CREC = [(Coleoptile length after regrowth) × 100] / (Coleoptile length of control 10 d after sowing)

CREG = (Coleoptile length after regrowth) - (Coleoptile length after cold treatment)

Table 1. Origin and subspecies of genotypes used in this study.

Number	Genotype	Origin	Subspecies
1	Diamante-INIA	Chile	Japonica
2	Zafiro-INIA	Chile	Japonica
3	Brillante-INIA	Chile	Japonica
4	Oro-INIA	Chile	Japonica
5	Quila 154601[1]	Chile	Japonica
6	Quila 154804[1]	Chile	Japonica
7	Quila 156603[1]	Chile	Japonica
8	Quila 157302[1]	Chile	Japonica
9	Quila 159005[1]	Chile	Japonica
10	Quila 173201[1]	Chile	Japonica
11	Quila 185007[1]	Chile	Japonica
12	Quila 216305[1]	Chile	Japonica
13	Quila 225001[1]	Chile	Japonica
14	Quila 225103[1]	Chile	Japonica
15	Quila 228603[1]	Chile	Japonica
16	Quila 230513[1]	Chile	Japonica
17	Quila 230601[1]	Chile	Japonica
18	Quila 230602[1]	Chile	Japonica
19	Quila 230603[1]	Chile	Japonica
20	Quila 231902[1]	Chile	Japonica
21	Quila 233008[1]	Chile	Japonica
22	Quila 235207[1]	Chile	Japonica
23	Quila 241309[1]	Chile	Japonica
24	Quila 242104[1]	Chile	Japonica
25	Guadiamar	Spain	Japonica
26	Karolina	Hungary	Japonica
27	Susan	Spain	Japonica
28	Chu Xian	China	Indica
29	Sugandh-2	India	Indica

[1]Experimental lines from the Rice Breeding Program of the Instituto de Investigaciones Agropecuarias (INIA).

Coleoptile length measured 7 d after sowing in cold conditions (CLEN) was also considered in the analysis.

Statistical analysis

Data were analyzed by one-way ANOVA. The factor was the genotypes. The LSD test ($P \leq 0.05$) was performed to detect differences between means. Three replicates were applied and each replicate represented a mean of 10 measurements of independent coleoptiles. Pearson's correlation coefficient was calculated for CLEN, CRED, and CREG. All analyses were performed with Infostat (Di Rienzo et al., 2011). Coleoptile reduction during cold treatment, coleoptile length after cold treatment, and coleoptile regrowth values were used for principal component analysis and cluster analysis. Principal component analysis with minimum spanning tree and cluster analysis were performed with the Infostat statistical software (Di Rienzo et al., 2011). The biplot was developed with the first two principal components (Gabriel, 1971). Cluster analysis was based on Ward's method using Euclidean distance.

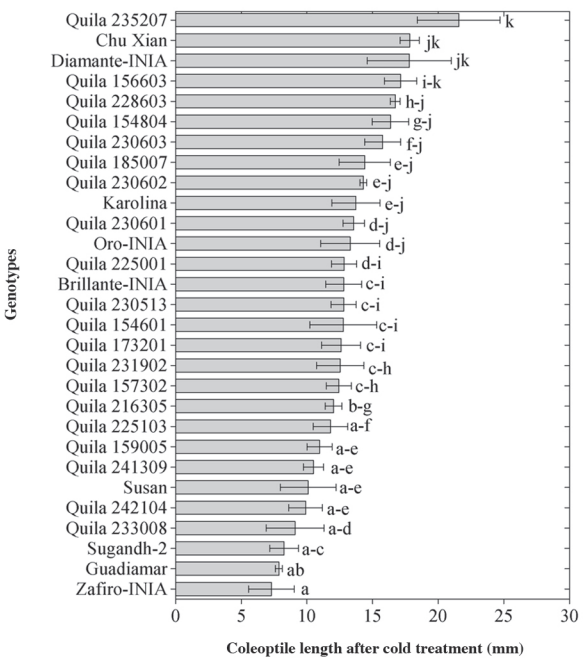
RESULTS AND DISCUSSION

Statistical analysis showed differences between rice genotypes for coleoptile length after cold treatment (CLEN), coleoptile reduction after cold treatment (CRED), and coleoptile regrowth (CREG). However, no

differences were found among genotypes when evaluating coleoptile recovery (CREC).

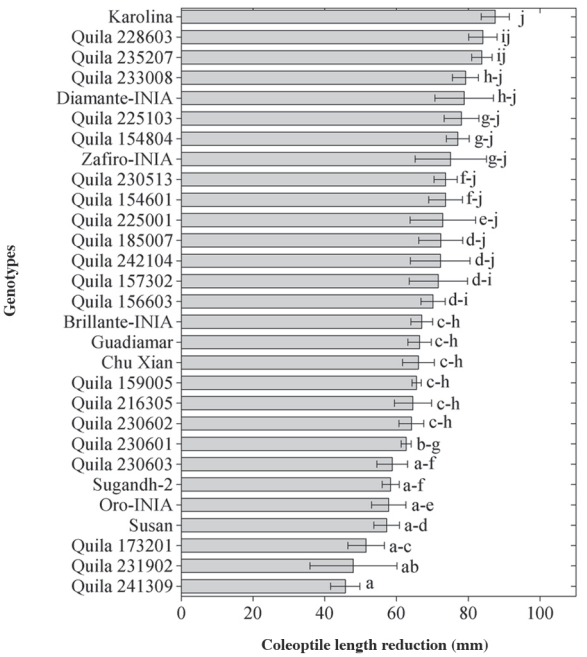
The coefficient of variance (CV) observed in CRED (14.0%) was lower than in CREG (18.9%), CLEN (21.5%), and CREC (22.5%). Coleoptile lengths were used to rank cold tolerance. Genotypes with high coleoptile length were considered as tolerant in the CLEN ranking (Figure 1). 'Quila 235207' (21.6 mm), 'Chu Xian' (17.8 mm), and 'Diamante-INIA' (17.8 mm) showed the highest coleoptile lengths after cold treatment and 'Zafiro-INIA' (7.3 mm) showed the lowest coleoptile length after cold treatment. Genotypes with a low reduction in coleoptile length were considered as cold tolerant in the CRED ranking (Figure 2). 'Karolina' (87.5 mm), 'Quila 228603' (84.1 mm), and 'Quila 235207' (83.8 mm) showed the highest reductions in coleoptile length during cold treatment. On the other hand, 'Quila 241309' (45.7 mm), 'Quila 231902' (47.9 mm), and 'Quila 173201' (51.5 mm) showed the lowest reductions in coleoptile length.

Genotypes with low values were considered as cold tolerant in the CREC ranking (Figure 3). This ranking did not show any differences between genotypes probably due to the high observed CV. In general, coleoptile length recoveries of all genotypes were between 50% and 100% of the control. 'Oro-INIA' exhibited the highest CREC value and 'Zafiro-INIA' the lowest. The CREC values were similar in other cultivars and experimental lines. Genotypes with high values were considered as cold



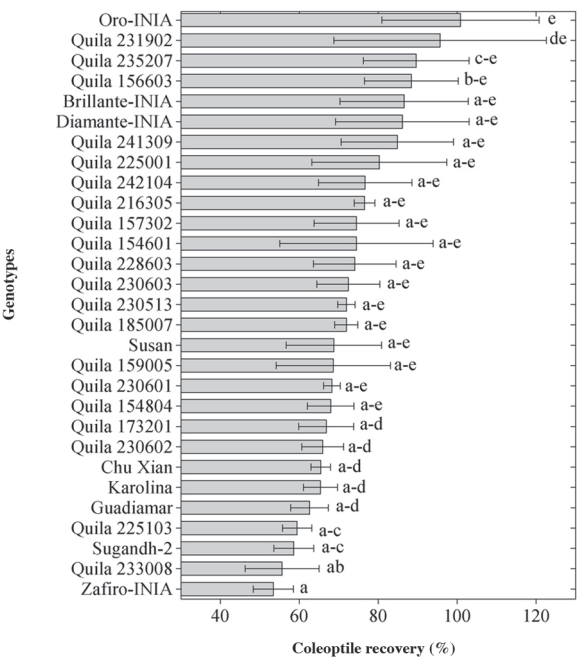
Each bar corresponds to the mean value of coleoptile length after cold treatment. The error bar represents the standard error of three replicates. Letters beside bars indicate differences between genotypes in cold treatment according to Fisher´s LSD test ($P \leq 0.05$).

Figure 1. Coleoptile length after cold treatment in 29 rice genotypes.



Each bar corresponds to the mean value of coleoptile length reduction after cold treatment. The error bar represents the standard error of three replicates. Letters beside bars indicate differences between genotypes in cold treatment according to Fisher´s LSD test ($P \leq 0.05$).

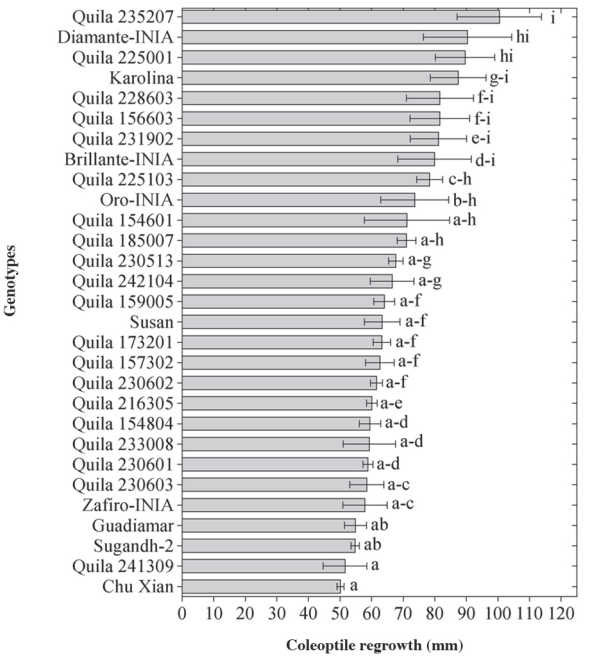
Figure 2. Coleoptile length reduction in 29 rice genotypes.



Each bar corresponds to the mean value of coleoptile length recovery compared with the control after regrowth. The error bar represents the standard error of three replicates. Letters beside bars indicate differences between genotypes according to Fisher´s LSD test ($P \leq 0.05$).

Figure 3. Coleoptile recovery in 29 rice genotypes.

tolerant in the CREG ranking (Figure 4),. 'Quila 235207' (100.4 mm) was the genotype with the highest CREG and 'Chu Xian' (50.1 mm) and 'Quila 241309' (51.6 mm) were the genotypes with the lowest. Both CREG and CLEN were highly positively correlated (Table 2) whereas CRED and CREG were lowly positively correlated. No correlation was observed between CLEN and CRED. The cold-tolerant genotype 'Diamante-INIA' showed high CRED, but exhibited a higher coleoptile length recovery after cold treatment and higher coleoptile growth under low temperatures than other rice cultivars. This result suggests that coleoptile reduction found in 'Diamante-INIA' could be compensated by high CLEN and high CREG. High CREG observed in 'Diamante-INIA', 'Oro-INIA', and 'Brillante-INIA' could be explained by more than 40 yr of adaption to low temperatures in Chile (Alvarado and Pino, 1982; Alvarado et al., 1997). 'Oro-INIA' was released in Chile in 1964, 'Diamante-INIA' as F_2 in 1962, and 'Brillante-INIA' contains genetic background from 'Diamante-INIA' and 'Oro-INIA' (Alvarado et al., 1997).



The bar represents the mean value of coleoptile regrowth. The error bar represents the standard error of three replicates. Letters beside bars indicate differences between genotypes in cold treatment according to Fisher´s LSD test ($P \leq 0.05$).

Figure 4. Coleoptile regrowth in 29 rice genotypes.

Table 2. Pearson's correlation coefficients between CRED, CREG, and CLEN traits.

Trait	CRED	CREG	CLEN
CRED	1.00	0.28*	0.14
CREG		1.00	0.56**
CLEN			1.00

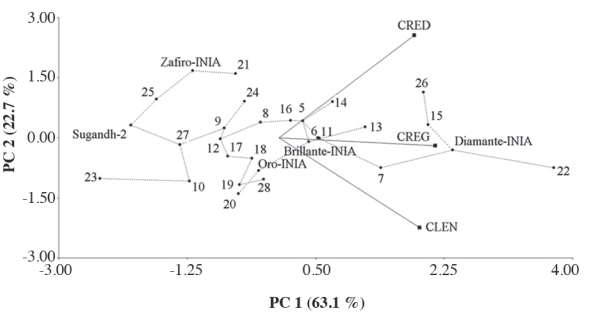
*$P \leq 0.05$, **$P \leq 0.001$

CRED: Coleoptile length reduction; CREG: coleoptile regrowth; CLEN: coleoptile length after cold treatment.

Another interesting result was a good correlation between CLEN and CREG values since CLEN is an easily measured trait and provides valuable information about the capacity of coleoptile emergence under cold conditions. An unexpected result was the positive correlation between CRED and CREG since several genotypes with a high growth capacity under cold temperatures had low growth after recovery, and vice versa. This result makes it more difficult to select genotypes for cold tolerance and analyzing different traits separately. Therefore, genotype selection was improved by using a combination of different traits in a biplot of principal components based on CRED, CLEN, and CREG traits (Figure 5).

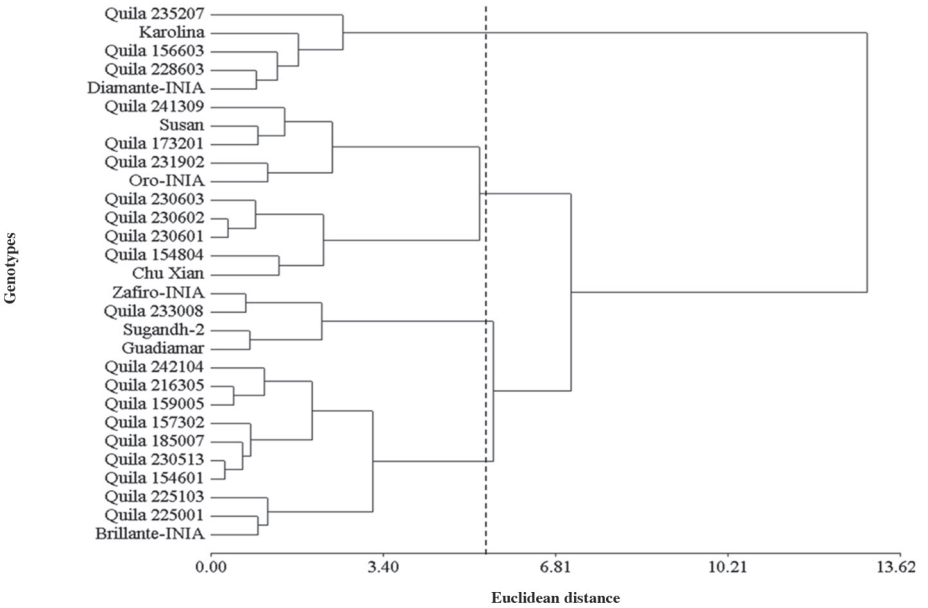
The principal components PC1 and PC2 represent 63.1% and 22.7% of total variability, respectively. Although PC1 discriminated among genotypes for cold tolerance, PC2 did not. The cold-tolerant genotype 'Diamante-INIA' had a positive value for PC1, and susceptible genotypes 'Sugandh-2' had a negative value for PC1 similar to 'Zafiro-INIA'. 'Brillante-INIA' and 'Oro-INIA' had an intermediate value for PC1 close to zero. The minimum spanning tree showed that cold tolerance of 'Diamante-INIA' was higher than other Chilean cultivars, such as 'Brillante-INIA' and 'Oro-INIA' with intermediate tolerance and 'Zafiro-INIA' with low tolerance. Furthermore, 'Quila 235207' exhibited the highest cold tolerance of the experimental lines from the Chilean breeding program and 'Quila 233008' had the lowest cold tolerance of all the experimental lines. Cluster analysis was performed for the same traits by grouping genotypes with different cold tolerances (Figure 6).

Four well-differentiated groups resulted from the analysis: first group, 'Diamante-INIA', 'Karolina' and three experimental lines (Table 3); second group, 'Oro-INIA', 'Chu Xian', 'Susan' and seven experimental lines; third group, 'Guadiamar', 'Sugandh-2', 'Zafiro-INIA' and one experimental line; and the fourth group, 'Brillante-INIA' and nine experimental lines. The mean of values



The biplot represents a principal component analysis between CLEN, CRED, and CREG of 29 genotypes. Numbers corresponding to genotypes are shown in Table 1. Dotted lines between squares (■) represent a Minimum Spanning Tree of the parameters.
PC: Principal component; CLEN: coleoptile length after cold treatment; CRED: coleoptile length reduction; CREG: coleoptile regrowth.

Figure 5. Biplot graph of cold tolerance evaluation in 29 rice genotypes based on coleoptile length.



Analysis of cluster similarity was based on Ward's method. Dotted line represents cutoff for definition of four groups.

Figure 6. Cluster analysis of 29 rice genotypes based on Euclidean distance using coleoptile length after cold treatment (CLEN), coleoptile length reduction (CRED), and coleoptile regrowth (CREG) traits.

for traits by group showed that the first group obtained the highest CRED (80.9 mm), CREC (88.3 mm), and CLEN (17.4 mm) values (Table 4). In contrast, CRED had a low value (69.8 mm); CREG (56.7 mm) and CLEN (8.1 mm) values were observed as the lowest in the third group. In general, cold treatment induced a decrease in coleoptile length in all the evaluated varieties and genotypes. The reduction of coleoptile growth under low temperatures observed in the present study was also observed by other authors (Castillo and Alvarado, 2002; Cruz and Milach, 2004; Sharifi and Aminpanah, 2010). Low temperatures affect germination in the activation stage and post-germination growth (Yoshida, 1981). Rice is susceptible to temperatures below 20 °C and does not have acclimation capacity at freezing temperatures. The optimum growth temperatures for rice are between 25 and 30 °C and the low critical temperature at the germination stage is 10 °C (Yoshida, 1981). Coleoptile length is fundamental for seedling establishment in a flooding condition (Luo et al., 2007). Low temperatures at the germination stage in Chile can inhibit or decrease coleoptile growth. Therefore, it is crucial for the Chilean Rice Breeding Program to identify, maintain, and improve germination under cold and germination regrowth after cold.

Table 3. Groups and genotypes resulting from cluster analysis.

Groups	Genotypes
I	Diamante-INIA, Karolina, Quila 156603, Quila 228603, and Quila 235207.
II	Chu Xian, Oro-INIA, Quila 154804, Quila 173201, Quila 230601, Quila 230602, Quila 230603, Quila 231902, Quila 241309, and Susan.
III	Guadiamar, Quila 233008, Sugandh-2, and Zafiro-INIA.
IV	Brillante-INIA, Quila 154601, Quila 157302, Quila 159005, Quila 185007, Quila 216305, Quila 225001, Quila 225103, Quila 230513, and Quila 242104.

Table 4. Mean values of coleoptile length reduction (CRED), coleoptile regrowth (CREG), and coleoptile length after cold treatment (CLEN) traits by groups.

Groups	CRED ± SD	CREG ± SD	CLEN ± SD
I	80.9 ± 9.4	88.3 ± 18.2	17.4 ± 4.2
II	58.9 ± 11.8	62.1 ± 12.2	13.7 ± 3.2
III	69.8 ± 11.9	56.7 ± 8.7	8.1 ± 2.3
IV	71.2 ± 9.4	71.1 ± 13.6	12.3 ± 2.3

SD: standard deviation.

CONCLUSIONS

Rice germination under low temperatures induced a decrease in coleoptile length growth in all evaluated genotypes. Genotype ranking, principal component, and cluster analysis based on coleoptile length allowed us to find differences in cold tolerance among rice genotypes. Of the Chilean varieties, 'Diamante-INIA' showed high cold tolerance, and 'Oro-INIA' and 'Brillante-INIA' showed an intermediate cold tolerance. By using this methodology, we found only three experimental lines with cold tolerance similar to 'Diamante-INIA', and all the other experimental lines exhibited intermediate to low cold tolerance. These results showed an interesting difference in the cold tolerance of some Chilean genotypes at the germination stage, thus confirming the need to evaluate the rest of the germplasm of the Rice Breeding Program.

ACKNOWLEDGEMENTS

We thank the Rice Breeding Program of the Instituto de Investigaciones Agropecuarias (INIA) for facilitating the germplasm. Part of this work was supported by the FONDECYT 1110405 Project.

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