Assessment of the genetic diversity and population structure in temperate *japonica* rice germplasm used in breeding in Chile, with SSR markers

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ABSTRACT

Rice Breeding Program (RBP) of the Instituto de Investigaciones Agropecuarias (INIA) at Chillán, Chile, has a rice (*Oryza sativa* L.) germplasm collection that consists of 1200 accessions, mainly temperate *japonica* rice accessions, well adapted to the local conditions. Most of the new introduced accessions adapt very poorly to Chilean agroecological conditions because of requirements of long days and cold tolerance. The objectives of this study were to use microsatellites to evaluate level of polymorphism of a representative sample of this collection and determine its genetic diversity and relationships with cultivated germplasm from different geographical origin. A total of 249 genotypes were analyzed with 30 selected polymorphic microsatellites. Total number of alleles scored across 249 genotypes was 183 with an overall mean of 6.1 alleles per locus, ranging 2-14. The mean major allele (most common) frequency was 0.61 and mean minor allele frequency was 0.028. The overall mean gene diversity across 30 SSR loci was 0.52. Mean heterozygosity was 0.01, and mean polymorphism information content (PIC) value was 0.47. The accessions were organized by structure analysis into three main groups and revealed a fairly consistent genetic relationship with dendrogram and Principal Coordinates Analysis (PCoA). The temperate *japonica* accessions can be further subdivided into three subpopulations where long and short Chilean varieties were grouped into different clusters. The three populations showed different level of admixture, admixture probably due to previous breeding work through years. Results indicate that polymorphism levels of Chilean rice temperate *japonica* collection has similar magnitude as temperate *japonica* germplasm reported in the literature.

Key words: Genetic diversity, microsatellites, *Oryza sativa*, rice, temperate *japonica* germplasm.

INTRODUCTION

The first attempts to introduce rice (*Oryza sativa* L.) in Chile dated from 1920, but it was until 1930 that farmers could cultivate rice in the country. The first germplasm introduced in the country by farmers and later on by the Ministry of Agriculture came from Asian and European countries. Currently, rice is produced in the area located between the Province of Linares (35°51' S) and the city of San Carlos (36°25') in the Province of Ñuble.

In Chile, rice is produced at the southern limit of its cultivation area; therefore, it suffers from low temperatures at the vegetative and flowering stages. Rice production occupies 23 714 ha with a total production of 163 560 Mg a mean yield of 6.9 Mg ha⁻¹ (ODEPA, 2015). The whole rice-growing area is under irrigation, using mainly the flooding system and with only a couple of thousands hectares sown with direct drill system. Cultivars grown in Chile are exclusively of the temperate *japonica* type with a length: width ratio close to 3.0 and are classified by the Chilean Norm as a long-width seeded type. Total per capita rice consumption is approximately 11 kg per year (ODEPA, 2015). Chile imports about 50% of domestic demand.

The Instituto de Investigaciones Agropecuarias (INIA) has managed for more than 50 yr the only existing rice breeding program (RBP) in the country. The main objective of this program is: to develop cultivars with tolerance to low temperature at the vegetative and reproductive stages, high grain and head yield potential, short growth duration, tolerance to lodging, and good quality. INIA has released eight rice varieties (Paredes et al., 2015).

The cultivated rice (*O. sativa*) can be divided into five distinct groups: *indica*, *aus*, *aromatic*, temperate *japonica*, and *tropical japonica* (Garris et al., 2005). A study of the USDA rice world collection indicated that *indica* and *aus* were highly diversified, while temperate and *tropical japonica* had the lower diversity. *Indica* and *aromatic* were genetically closer to *tropical japonica* than to temperate *japonica* (Garris et al., 2005; Agrama et al., 2010). In spite of the richness of genetic variations of *indica*, *aus*, *aromatic*, and *tropical japonica* cultivars their presence in the Chilean rice collection is very limited due to their poor adaption to the local conditions such as a long-day and cold tolerance. These constraints do not have allowed the usage of this germplasm to broaden the genetic base of the temperate *japonica* accessions grown in the country.

Demand for high productivity and homogeneity in the new varieties has resulted in high-yielding varieties with a narrow genetic diversity (Becerra et al., 2015). Then, breeding new rice cultivars adapted to the local conditions that combine high yield potential, good grain quality, and resistance to both biotic and

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abiotic stress is a challenge and is urgently needed to meet future consumer demands (Liakat-Ali et al., 2011). To accomplish this task, plant breeding needs the presence of genetic variation, a better understanding of the population structure, combined with the use of efficient selection strategies to exploit the existing genetic resources (Lu et al., 2005; Zhang et al., 2011). In spite of the importance of this information for the rice breeding program, there is not a complete study on the temperate *japonica* rice germplasm utilized in the country, at molecular level.

Currently, there are different types of molecular markers available for assessing genetic diversity in crop species. Among them, simple sequence repeats (SSRs) or microsatellites are very useful for analyzing the structure of germplasm collections since they are abundant, co-dominant, multi-allelic, highly polymorphic, chromosome specific and easy genotype by PCR (Jamil et al., 2013).

The SSR markers are particularly suitable for evaluating genetic diversity and relationships among plant species, populations, or individuals (Tu et al., 2007), germplasm conservation or utilization (Sharma et al., 2007); marker-assisted selection (Rani and Adilakshmi, 2011); cultivar identification; hybrid purity analysis, gene mapping studies (Sarao et al., 2010), and parents selection in breeding programs (Xu et al., 2002). In rice, SSR markers have been

widely used in assessing genetic diversity (Agrama et al., 2010; Courtois et al., 2012; Zhang et al., 2012).

The aim of this study was to analyze a representative sample of the temperate *japonica* rice germplasm utilized in Chile by the RBP, using microsatellites, in order to provide information of the level of genetic diversity, as well as, a better understanding of its organization for a future management and exploitation.

MATERIALS AND METHODS

Plant material

Two hundred and forty nine well adapted rice accessions were selected for genotyping from the Rice Breeding Program (RBP). The entire germplasm collection was stratified by its morphological and phenological data available. The sampling strategy was systematic and random to represent the diversity of the collection. This representative sample represented about 20% of the current RBP working collection. Most of this germplasm is temperate *japonica* and included commercial and old varieties, experimental lines, and four non *japonica* types, two *aromatic* accessions (Basmati) and two scented accessions (Sugandh) as out group (Table 1) for the analysis. Seeds were provided by INIA's RBP at Chillán, Chile.

Table 1. Temperate *japonica* rice accessions evaluated by SSR.

N°	Accession	Origin	Subspecies	Grain type	Structure
1	Oro*	Chile	<i>japonica</i>	Short	Group3
2	Ambar-INIA*	Chile	<i>japonica</i>	Short	Group1
3	Quella-INIA*	Chile	<i>japonica</i>	Short	Group3
4	Brillante-INIA*	Chile	<i>japonica</i>	Long	Group2
5	Buli-INIA*	Chile	<i>japonica</i>	Long	Group2
6	Diamante-INIA*	Chile	<i>japonica</i>	Long	Group2
7	Cuarzo-INIA*	Chile	<i>japonica</i>	Long	Group2
8	Zafiro-INIA*	Chile	<i>japonica</i>	Long	Group2
9	CINIA 609	Chile	<i>japonica</i>	Long	Group2
10	Quila 157302	Chile	<i>japonica</i>	Short	Group2
11	Quila 154601	Chile	<i>japonica</i>	Medium	Group3
12	Quila 154804	Chile	<i>japonica</i>	Medium	Group3
13	Quila 156603	Chile	<i>japonica</i>	Medium	Group3
14	Quila 159005	Chile	<i>japonica</i>	Short	Group2
15	Quila 173201	Chile	<i>japonica</i>	Medium	Group3
16	Quila 185007	Chile	<i>japonica</i>	Medium	Group3
17	Quila 213801	Chile	<i>japonica</i>	Medium	Group3
18	Quila 216305	Chile	<i>japonica</i>	Long	Group3
19	Quila 221801	Chile	<i>japonica</i>	Long	Group3
20	Quila 225001	Chile	<i>japonica</i>	Medium	Group3
21	Quila 225101	Chile	<i>japonica</i>	Medium	Group3
22	Quila 225103	Chile	<i>japonica</i>	Long	Group3
23	Quila 228603	Chile	<i>japonica</i>	Long	Group3
24	Quila 230513	Chile	<i>japonica</i>	Extra Large	Group1
25	Quila 230601	Chile	<i>japonica</i>	Long	Group3
26	Quila 230602	Chile	<i>japonica</i>	Long	Group3
27	Quila 230603	Chile	<i>japonica</i>	Long	Group3
28	Quila 231902	Chile	<i>japonica</i>	Medium	Group3
29	Quila 233008	Chile	<i>japonica</i>	Medium	Group3
30	Quila 234801	Chile	<i>japonica</i>	Long	Group1
31	Quila 235207	Chile	<i>japonica</i>	Long	Group3
32	Quila 235501	Chile	<i>japonica</i>	Long	Group2
33	Quila 237908	Chile	<i>japonica</i>	Long	Group3
34	Platino-INIA*	Chile	<i>japonica</i>	Short	Group1
35	Quila 242104	Chile	<i>japonica</i>	Short	Group1

N°	Accession	Origin	Subspecies	Grain type	Structure
36	Corea 2	Corea	<i>japonica</i>	Short	Group3
37	Guadamar	España	<i>japonica</i>	Short	Group1
38	Guara	España	<i>japonica</i>	Medium	Group3
39	Hispagran	U.S.A	<i>japonica</i>	Medium	Group3
40	Susan	España	<i>japonica</i>	Medium	Group3
41	Euro	Europa	<i>japonica</i>	Medium	Group3
42	Ranballi	Bulgaria	<i>japonica</i>	Short	Group1
43	Karolina	Hungría	<i>japonica</i>	Long	Group3
44	Basmati C621	India	<i>aromatic</i>	Long	Group2
45	Basmati	India	<i>aromatic</i>	Long	Group3
46	Sugandh-2	India	<i>aromatic</i>	Extra Large	Group3
47	Sugandh-3	India	<i>aromatic</i>	Extra Large	Group3
48	Chu Xiang	China	<i>japonica</i>	Medium	Group3
49	Arroz Negro	Brasil	<i>japonica</i>	Medium	Group3
50	IRRI-Li Jian-X-H	China	<i>japonica</i>	Extra Large	Group3
51	IRRI-Yuhkara	Japón	<i>japonica</i>	Extra Large	Group3
52	Quila 213007	Chile	<i>japonica</i>	Medium	Group3
53	Rquila 17	Chile	<i>japonica</i>	Long	Group2
54	Quila 242101	Chile	<i>japonica</i>	Short	Group3
55	Quila 222704	Chile	<i>japonica</i>	Long	Group3
56	Quila 208902	Chile	<i>japonica</i>	Long	Group2
57	Quila 216501	Chile	<i>japonica</i>	Long	Group2
58	Quila 194603	Chile	<i>japonica</i>	Long	Group2
59	Quila 200112	Chile	<i>japonica</i>	Medium	Group3
60	Quila 242610	Chile	<i>japonica</i>	Short	Group1
61	Quila 242207	Chile	<i>japonica</i>	Long	Group1
62	INIAG 70	Chile	<i>japonica</i>	Long	Group2
63	Quila 242420	Chile	<i>japonica</i>	Medium	Group1
64	Quila 241319	Chile	<i>japonica</i>	Medium	Group1
65	Quila 242616	Chile	<i>japonica</i>	Short	Group1
66	Quila 194602	Chile	<i>japonica</i>	Long	Group2
67	Quila 222703	Chile	<i>japonica</i>	Long	Group3
68	Quila 240101	Chile	<i>japonica</i>	Long	Group2
69	Quila INIAG 152	Chile	<i>japonica</i>	Long	Group2
70	Quila 224802	Chile	<i>japonica</i>	Long	Group2

Continuation Table 1.

N°	Accession	Origin	Subspecies	Grain type	Structure	N°	Accession	Origin	Subspecies	Grain type	Structure
71	INIAG 144	Chile	japonica	Long	Group2	138	Quila 242012	Chile	japonica	Short	Group1
72	Quila 216202	Chile	japonica	Long	Group2	139	INIAG 27	Chile	japonica	Short	Group1
73	INIAG 115	Chile	japonica	Long	Group2	140	Quila 241605	Chile	japonica	Short	Group1
74	Quila 242703	Chile	japonica	Long	Group2	141	Quila 242106	Chile	japonica	Short	Group3
75	Rquila 363	Chile	japonica	Long	Group2	142	Quila 242108	Chile	japonica	Short	Group1
76	INIAG 99	Chile	japonica	Long	Group2	143	Quila 241701	Chile	japonica	Short	Group2
77	Rquila 205	Chile	japonica	Long	Group2	144	Quila 241607	Chile	japonica	Short	Group1
78	INIAG 169	Chile	japonica	Long	Group2	145	Quila 241321	Chile	japonica	Short	Group1
79	INIAG 165	Chile	japonica	Long	Group2	146	Quila 241304	Chile	japonica	Short	Group1
80	Quila 249006	Chile	japonica	Long	Group1	147	Quila 249101	Chile	japonica	Short	Group1
81	Quila 249104	Chile	japonica	Long	Group2	148	Quila 249103	Chile	japonica	Short	Group1
82	Quila 249203	Chile	japonica	Long	Group2	149	Quila 249303	Chile	japonica	Short	Group1
83	Quila 223105	Chile	japonica	Extra Large	Group3	150	Quila 249304	Chile	japonica	Short	Group1
84	Quila 231701	Chile	japonica	Extra Large	Group3	151	Quila 251303	Chile	japonica	Short	Group3
85	Quila 242203	Chile	japonica	Medium	Group2	152	Quila 252702	Chile	japonica	Medium	Group3
86	Quila 222204	Chile	japonica	Long	Group2	153	Quila 252201	Chile	japonica	Short	Group1
87	Quila 242002	Chile	japonica	Long	Group2	154	Quila 242204	Chile	japonica	Short	Group1
88	Rquila 28	Chile	japonica	Long	Group3	155	Quila 242609	Chile	japonica	Short	Group1
89	INIAG 79	Chile	japonica	Long	Group2	156	Quila 242612	Chile	japonica	Short	Group1
90	Quila 256603	Chile	japonica	Long	Group2	157	Quila 242112	Chile	japonica	Short	Group1
91	Quila 256001	Chile	japonica	Long	Group2	158	Quila 242613	Chile	japonica	Short	Group1
92	Quila 256002	Chile	japonica	Long	Group2	159	Quila 241307	Chile	japonica	Medium	Group1
93	Quila 256602	Chile	japonica	Long	Group2	160	Quila 242608	Chile	japonica	Short	Group1
94	Quila 251702	Chile	japonica	Long	Group3	161	Quila 242808	Chile	japonica	Short	Group1
95	Quila 256601	Chile	japonica	Long	Group2	162	Quila 242121	Chile	japonica	Short	Group1
96	Quila 256501	Chile	japonica	Long	Group2	163	Quila 242114	Chile	japonica	Short	Group1
97	Quila 252801	Chile	japonica	Long	Group2	164	Quila 256104	Chile	japonica	Short	Group1
98	Quila 254101	Chile	japonica	Long	Group2	165	Quila 256103	Chile	japonica	Short	Group1
99	Quila 244013	Chile	japonica	Long	Group2	166	Quila 256106	Chile	japonica	Short	Group1
100	Quila 241801	Chile	japonica	Medium	Group1	167	Quila 256101	Chile	japonica	Short	Group1
101	Quila 241612	Chile	japonica	Medium	Group2	168	Quila 256701	Chile	japonica	Short	Group3
102	Quila 242206	Chile	japonica	Medium	Group1	169	Quila 256901	Chile	japonica	Short	Group2
103	Quila 243008	Chile	japonica	Short	Group1	170	Quila 256903	Chile	japonica	Short	Group1
104	Quila 242010	Chile	japonica	Short	Group2	171	Quila 256902	Chile	japonica	Short	Group2
105	Quila 223202	Chile	japonica	Medium	Group3	172	Quila 249201	Chile	japonica	Medium	Group1
106	Quila 241610	Chile	japonica	Medium	Group2	173	Quila 249501	Chile	japonica	Medium	Group3
107	Quila 241703	Chile	japonica	Short	Group2	174	Quila 251703	Chile	japonica	Medium	Group2
108	Quila 225105	Chile	japonica	Medium	Group3	175	Quila 252701	Chile	japonica	Medium	Group3
109	Quila 242011	Chile	japonica	Medium	Group2	176	Quila 256401	Chile	japonica	Medium	Group3
110	RQuila 356	Chile	japonica	Medium	Group3	177	Quila 256604	Chile	japonica	Medium	Group2
111	Quila 251301	Chile	japonica	Extra Large	Group3	178	Quila 256402	Chile	japonica	Medium	Group2
112	Quila 254102	Chile	japonica	Extra Large	Group3	179	Quila 256605	Chile	japonica	Medium	Group2
113	Quila 254701	Chile	japonica	Extra Large	Group3	180	Quila 256801	Chile	japonica	Medium	Group2
114	Quila 253701	Chile	japonica	Extra Large	Group2	181	Quila 260405	Chile	japonica	Medium	Group3
115	Quila 260312	Chile	japonica	Long	Group3	182	Quila 240102	Chile	japonica	Medium	Group3
116	Quila 260404	Chile	japonica	Long	Group3	183	Quila 241403	Chile	japonica	Medium	Group1
117	Quila 242003	Chile	japonica	Medium	Group2	184	Quila 240203	Chile	japonica	Medium	Group1
118	Quila 242701	Chile	japonica	Medium	Group2	185	Quila 244104	Chile	japonica	Medium	Group1
119	Quila 242006	Chile	japonica	Medium	Group2	186	Quila 243306	Chile	japonica	Medium	Group1
120	Quila 242504	Chile	japonica	Medium	Group2	187	Quila 245801	Chile	japonica	Short	Group3
121	Quila 242115	Chile	japonica	Short	Group1	188	Quila 243201	Chile	japonica	Short	Group1
122	Quila 242007	Chile	japonica	Medium	Group2	189	Quila 220001	Chile	japonica	Medium	Group2
123	Quila 242802	Chile	japonica	Medium	Group2	190	Quila 257501	Chile	japonica	Short	Group1
124	Quila 240103	Chile	japonica	Medium	Group3	191	Quila 257003	Chile	japonica	Short	Group1
125	Quila 240204	Chile	japonica	Medium	Group1	192	Quila 251302	Chile	japonica	Short	Group1
126	Quila 240208	Chile	japonica	Medium	Group1	193	Quila 257502	Chile	japonica	Short	Group1
127	Quila 241606	Chile	japonica	Medium	Group1	194	Quila 249005	Chile	japonica	Short	Group1
128	Quila 249301	Chile	japonica	Short	Group1	195	Quila 249305	Chile	japonica	Short	Group1
129	Quila 243010	Chile	japonica	Medium	Group3	196	Quila 241402	Chile	japonica	Medium	Group1
130	Quila 241305	Chile	japonica	Medium	Group1	197	Quila 243901	Chile	japonica	Medium	Group2
131	Quila 241313	Chile	japonica	Short	Group1	198	Quila 241608	Chile	japonica	Short	Group2
132	Quila 225102	Chile	japonica	Medium	Group3	199	Quila 242617	Chile	japonica	Short	Group1
133	Quila 242415	Chile	japonica	Medium	Group2	200	Quila 243102	Chile	japonica	Short	Group1
134	Quila 253003	Chile	japonica	Short	Group1	201	Quila 244012	Chile	japonica	Short	Group1
135	Quila 249002	Chile	japonica	Medium	Group3	202	Quila 244501	Chile	japonica	Short	Group1
136	Quila 240201	Chile	japonica	Short	Group1	203	Quila 243304	Chile	japonica	Short	Group3
137	Quila 241315	Chile	japonica	Medium	Group1	204	Quila 242118	Chile	japonica	Short	Group3

Continuation Table 1.

N°	Accession	Origin	Subspecies	Grain type	Structure
205	Quila 246901	Chile	<i>japonica</i>	Medium	Group3
206	Quila 241312	Chile	<i>japonica</i>	Short	Group1
207	Quila 242008	Chile	<i>japonica</i>	Short	Group2
208	INIAG 220	Chile	<i>japonica</i>	Long	Group2
209	Quila 238803	Chile	<i>japonica</i>	Long	Group3
210	Quila 238908	Chile	<i>japonica</i>	Long	Group3
211	Quila 242004	Chile	<i>japonica</i>	Long	Group2
212	Quila 242005	Chile	<i>japonica</i>	Medium	Group2
213	Quila 242205	Chile	<i>japonica</i>	Short	Group1
214	Quila 257004	Chile	<i>japonica</i>	Short	Group1
215	Quila 257301	Chile	<i>japonica</i>	Short	Group1
216	Quila 258301	Chile	<i>japonica</i>	Short	Group1
217	Quila 261601	Chile	<i>japonica</i>	Long	Group3
218	Quila 261801	Chile	<i>japonica</i>	Long	Group3
219	Quila 261803	Chile	<i>japonica</i>	Long	Group3
220	Quila 262003	Chile	<i>japonica</i>	Short	Group3
221	Quila 262101	Chile	<i>japonica</i>	Long	Group2
222	Quila 262102	Chile	<i>japonica</i>	Long	Group2
223	Quila 262103	Chile	<i>japonica</i>	Extra Large	Group3
224	Quila 262104	Chile	<i>japonica</i>	Extra Large	Group3
225	Quila 262301	Chile	<i>japonica</i>	Short	Group3
226	Quila 262401	Chile	<i>japonica</i>	Long	Group3
227	Quila 262602	Chile	<i>japonica</i>	Short	Group3

N°	Accession	Origin	Subspecies	Grain type	Structure
228	Quila 262802	Chile	<i>japonica</i>	Long	Group3
229	Quila 262803	Chile	<i>japonica</i>	Long	Group3
230	Quila 263102	Chile	<i>japonica</i>	Long	Group3
231	Quila 263304	Chile	<i>japonica</i>	Long	Group3
232	Quila 263901	Chile	<i>japonica</i>	Short	Group3
233	Quila 264001	Chile	<i>japonica</i>	Long	Group2
234	Quila 264601	Chile	<i>japonica</i>	Short	Group3
235	Quila 265105	Chile	<i>japonica</i>	Medium	Group2
236	Quila 265602	Chile	<i>japonica</i>	Long	Group3
237	Quila 266001	Chile	<i>japonica</i>	Short	Group3
238	Quila 266002	Chile	<i>japonica</i>	Short	Group2
239	Quila 266405	Chile	<i>japonica</i>	Short	Group3
240	Quila 266502	Chile	<i>japonica</i>	Long	Group3
241	Inca	Colombia	<i>japonica</i>	Long	Group2
242	Sha-Tiao-Tsao	China	<i>japonica</i>	Medium	Group3
243	Alinamo. C.	Colombia	<i>japonica</i>	Long	Group3
244	CT6742-12-CA-32	Colombia	<i>japonica</i>	Extra Large	Group2
245	H404	Hungría	<i>japonica</i>	Medium	Group3
246	Fanny	Francia	<i>japonica</i>	Medium	Group3
247	Quila 154907	Chile	<i>japonica</i>	Extra Large	Group2
248	CT6750-9-2-4-2-M-M-3	Colombia	<i>japonica</i>	Long	Group2
249	PRA557	Madagascar	<i>japonica</i>	Extra Large	Group3

Genotypic characterization

Germination and plant vegetative growth of the accessions were carried out under greenhouse conditions. Harvested leaves at the 4 leaf-stage were maintained at -86 °C until genomic DNA was extracted. Leaves were macerated with liquid nitrogen and homogenized with DNA extraction buffer (100 mM Trizma; 1.4 M NaCl; 20 mM EDTA; 1% polyvinylpyrrolidone; 2% CTAB; 1% β -mercaptoethanol; pH 8.0). Samples were incubated for 1 h at 65 °C, and followed by two protein extractions with chloroform-isoamyl alcohol (24:1). The mix was centrifuged at 5000 rpm for 15 min and DNA was precipitated with isopropanol at -20 °C overnight. DNA pellet was washed with ethanol (70% and 95%), dried at room temperature, resuspended in TE buffer (pH 8.0) and treated with RNase.

DNA quality was verified by electrophoresis, in a 1% agarose gel with 1×TAE buffer at 100 V and genomic DNA size was compared to λ HindIII ladder. Finally, DNA concentration was measured in a UV-Vis spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Wilmington, Delaware, USA) and each accession sample was diluted to a 5 ng μ L⁻¹ concentration.

Microsatellite (SSR) selection and evaluation

A set of 200 nuclear SSR markers, distributed among the 12 rice chromosomes (<http://www.gramene.org/markers>), were initially screened to evaluate genetic diversity on five randomly selected rice accessions (Oro, 'Zafiro-INIA', Susan, Rquila28 and Quila253701). Finally, 30 SSRs were selected on their performance, level of polymorphism, and reproducibility for genetic analysis of the 249 rice accessions (Table 2).

The PCR reaction conditions were performed in a 12.5 μ L total volume made up of 0.1 μ M of each primer, 1 unit of *Taq* DNA polymerase, 0.2 μ M of each dNTP, 10 mM Tris-HCl pH 7.2, 50 mM KCl, 1.5 mM MgCl₂, DMSO (50%), and 10 ng DNA. The reaction was amplified in DNA Engine Dyad Thermal Cycler (Bio-Rad Laboratories, Alameda, California, USA) programmed for one cycle at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, 55-65 °C (in accordance with the primer) for 2 min, and an extension period at 72 °C for 7 min.

Amplification products were mixed with a loading buffer and denatured at 96 °C for 4 min. A 1.5 μ L aliquot of PCR products was loaded onto 6% denaturing polyacrylamide gels and run in 0.5X TBE buffer at 1800 V for approximately 2 h. Silver staining was performed to visualize DNA fragments according to Promega's protocol (Promega Corporation, Madison, Wisconsin, USA). The fragment sizes were estimated based on Perfect DNA 50 bp (Calbiochem, Merck, Darmstadt, Germany) and 50-2000 bp (Novagen, Merck) ladders. The stained gel was dried and documented by using a scanner.

Allele scoring and data analysis

For each SSR primer, the amplified SSR alleles were scored based on their presence or absence. The resulting genotype matrix was analyzed for genetic diversity parameters and population structure.

Based on the observed alleles, the PowerMarker software v.3.25 (Lui and Muse, 2005), was used to calculate the following diversity parameters: allele number per *locus*, major and minor allele frequency and standard deviation of alleles, genetic diversity (He), heterozygosity, polymorphism information content (PIC).

Table 2. SSRs used to fingerprint temperate *japonica* rice accessions.

SSR Marker	Chromosome	Forward primer sequence	Reverse primer sequence	Annealing temperature (°C)
RM1164	3	CGTTTCTCCGAGAAAAGTCG	CAAGGTGGTCGTTGAGGC	55
RM8068	1	AAACCTCTCGCTGTAAATTAG	TGAACATTTATTGATATGGTAAA	57
RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	53
RM286	11	GGCTTCATCTTTGGCGAC	CCGGATTACAGAGATAAACTC	55
RM10	7	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC	55
RM502	8	GCGATCGATGGCTACGAC	ACAACCCAACAAGAAGGACG	55
RM21	11	ACAGTATTCCTGAGGCACGG	GCTCCATGAGGGTGGTAGAG	55
OSR28	9	AGCAGCTATAGCTTAGCTGG	ACTGCACATGAGCAGAGACA	55
RM44	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCTTACC	53
RM276	6	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	55
RM259	1	TGGAGTTTGAGAGGAGGG	CTTGTGTCATGGTGCCATGT	55
RM1230	3	GGGTGGTGTGAGCTTTTCTC	TTCCACTTCGACAACCCCTTC	55
RM482	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC	55
RM537	4	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC	55
RM547	8	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG	55
RM561	2	GAGCTGTTTTGGACTACGGC	GAGTAGCTTTTCTCCACCCC	55
RM560	7	GCAGGAGAACAGAAATCAGC	AGCCCGTGATACGGTGATAG	55
RM1261	12	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	55
RM241	4	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTAGTG	55
RM426	3	ATGAGATGAGTTCAAGGCC	AACTCTGTACCTCCATCGCC	55
RM17	-	TGCCCTGTATTTCTTCTCTC	GGTGATCCTTTCCCAITTTCA	55
RM243	-	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	55
RM406	2	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG	55
RM447	8	CGGTGTGTAAACTCGAAGCACC	TGCCGTGGCTCATTAGTGGTC	55
RM509	5	TAGTGAGGGAGTGGAACGG	ATCGTCCCCACAATCTCATC	55
RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTTC	55
RM525	2	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	55
RM555	2	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC	55
RM583	1	AGATCCATCCCTGTGGAGAG	GCGAACTCGCGTTGTAATC	55
RM5463	6	ACCCTTGACAGACAACGTACC	ATATACCAGCAGCTGCATGC	55

In order to understand the genetic structure, a genetic distance based approach and a model based approach were used. First, genetic similarity between pairs was estimated by Jaccard's coefficient with the SIMQUAL option. The similarity matrix was run on sequential, agglomerative, hierarchical, and nested clustering (SAHN) (Sneath and Sokal, 1973) using the unweighted pair-group method with arithmetic average (UPGMA) clustering algorithm to generate a dendrogram. The CPH option was used to generate a matrix of cophenetic values. This matrix was used in the MXCOMP option to calculate the correlation between the cophenetic matrix and the original matrix being clustered (SIMQUAL). This analysis measured goodness-of-fit in fewer than 1000 permutations and provided a cophenetic correlation value (r). A cophenetic correlation value of $r > 0.9$ is considered a very good fit according to Mantel (1967). To determine the association among the accessions, unweighted pair group method with arithmetic mean (UPGMA) tree was done. All computations were done with the NTSYSpc 2.1 program (Rohlf, 2000).

Also, the coordinates for each accession calculated in the Principal Coordinates Analysis (PCoA) were plotted using the Graph module and the G3D procedure of the software program SAS (SAS Institute, Cary, North Carolina, USA).

For the model based approach, Structure ver. 2.3.4 software was used (Pritchard et al., 2000). The number of subpopulations (K) was identified by this method. For each run, the admixture model, without prior information,

was applied with a burn-in period of 100 000, followed by a 100 000 Monte Carlo Markov Chain (MCMC) replicates. Each k value was run 10 times with k value varying from 2 to 8. True number of subpopulations was identified using the maximum value of $L(K)$. The optimum k value was determined as Evanno et al. (2005).

RESULTS

Genetic diversity

A total of 30 polymorphic microsatellites were selected and used to evaluate the total of 249 rice accessions (Table 2). The total number of alleles scored across the 249 genotypes was 183 with an overall mean of 6.1 alleles per locus ranging from 2 to 14 (Table 3, Figure 1). The most polymorphic loci were RM8068 (14 alleles), RM286 and RM44 (11 alleles), and RM547 (10 alleles). In contrast, the least polymorphic loci were RM17 (2 alleles) and RM502, RM482, RM561, RM509, and RM555 (3 alleles).

The mean major allele (most common) frequency was 0.61 and the RM555 marker exhibited the highest major allele frequency. The mean minor allele frequency was 0.028 and the RM1261 and RM243 markers had the lowest minor allele frequencies. The mean number of genotypes was 7.03 and RM8068 detected the highest number of genotypes (17.0) followed by RM44 (15.0). The overall mean gene diversity or expected heterozygosity (H_e) across the 30 SSR loci was 0.52. The highest gene diversity (0.73) was

Table 3. SSR marker, allele number, major and minor frequencies and their standard deviation (SD), genotype number, gene diversity, heterozygosity, and polymorphism information content (PIC).

SSR Marker	Allele number	Major allele frequency	SD	Minor allele frequency	SD	Genotype number	Gene diversity (He)	Heterozygosity	PIC
RM1164	5	0.62	0.031	0.004	0.004	5	0.51	0.00	0.43
RM8068	14	0.61	0.031	0.004	0.004	17	0.61	0.02	0.60
RM413	4	0.60	0.031	0.004	0.004	5	0.51	0.02	0.41
RM286	11	0.58	0.031	0.012	0.007	13	0.64	0.01	0.62
RM10	6	0.46	0.032	0.008	0.006	6	0.62	0.00	0.55
RM502	3	0.79	0.026	0.004	0.004	4	0.34	0.02	0.28
RM21	6	0.77	0.026	0.008	0.006	7	0.39	0.01	0.37
OSR28	8	0.48	0.032	0.004	0.004	12	0.67	0.03	0.62
RM44	11	0.44	0.031	0.004	0.004	15	0.73	0.03	0.70
RM276	5	0.77	0.027	0.012	0.007	5	0.39	0.00	0.36
RM259	7	0.52	0.032	0.004	0.004	7	0.63	0.00	0.57
RM1230	5	0.90	0.019	0.004	0.004	5	0.19	0.00	0.19
RM482	3	0.58	0.032	0.034	0.012	3	0.51	0.00	0.41
RM537	4	0.76	0.027	0.008	0.006	4	0.37	0.00	0.32
RM547	10	0.62	0.031	0.004	0.004	11	0.59	0.01	0.56
RM561	3	0.46	0.031	0.190	0.025	5	0.63	0.04	0.55
RM560	4	0.47	0.031	0.020	0.009	5	0.61	0.05	0.53
RM1261	7	0.55	0.031	0.002	0.002	8	0.60	0.02	0.54
RM241	10	0.42	0.031	0.016	0.008	10	0.73	0.00	0.69
RM426	8	0.67	0.030	0.004	0.004	8	0.53	0.00	0.51
RM17	2	0.64	0.031	0.357	0.031	2	0.46	0.00	0.35
RM243	7	0.47	0.032	0.002	0.002	8	0.64	0.01	0.57
RM406	6	0.81	0.025	0.008	0.006	6	0.33	0.00	0.31
RM447	4	0.85	0.023	0.008	0.006	4	0.26	0.00	0.24
RM509	3	0.77	0.027	0.036	0.012	3	0.36	0.00	0.32
RM510	5	0.52	0.032	0.008	0.006	5	0.52	0.00	0.41
RM525	7	0.42	0.031	0.012	0.007	10	0.71	0.04	0.66
RM555	3	0.87	0.021	0.028	0.010	3	0.24	0.00	0.22
RM583	7	0.49	0.032	0.004	0.004	8	0.69	0.00	0.65
RM5463	5	0.47	0.032	0.021	0.009	7	0.62	0.03	0.54
Mean	6.1	0.61		0.028		7.03	0.52	0.01	0.47

Figure 1. Banding pattern obtained in *Oryza sativa* with SRR OSR28.

detected by RM44 and RM241. The mean heterozygosity was very low (0.01) and the highest value was detected by RM560 (0.05) and several *loci* did not detect any level of heterozygosity. The PIC value for the SSR *loci* ranged from 0.19 (RM1230) to 0.70 (RM44) with a mean of 0.47 across of the 30 *loci* (Table 3), these PIC values represent the relative informativeness of each SSR marker in this study.

Germplasm structure

A neighbor-joining tree of 249 accession based on Jaccard's coefficient grouped accessions into two main groups, the temperate *japonica* (243 genotypes) and the non *japonica* type (6 genotypes). The non *japonica* genotypes were represented by *aromatic* (1 genotypes) and *scented* (5 genotypes) rice genotypes (Figure 2). Most of the 249

Figure 2. Dendrogram of genetic diversity of 249 temperate *japonica* rice accessions using 30 SSR markers (Jaccard's coefficient).

analyzed genotypes were distinguished by the 30 SSRs, with the exception of the following group of genotypes: INIA27-Quila256101, INIAG152-INIAG144, RQuila636-INIAG99-INIA169-INIAG165, Quila230602-Quila230603, Quila194603-Quila194602, and Sungandh-2-Sugandh-3 (Figure 2). The cophenetic correlation coefficient between the cophenetic matrix and the original SSR data was 0.80. This high value revealed that the original matrix is well represented in the dendrogram.

Cluster I represented by 243 temperate *japonica* sample was subdivided into two main subgroups. Based on the information of the type of grain. The subgroup I included 66 genotypes mainly short (76%) and medium (20%) grain accessions (length: width ratio of 2.0). This cluster also included the Chilean short-seeded type cultivars Oro, Quella-INIA and Ámbar-INIA.

On the other hand, the subgroup II included 178 genotypes, composed with long (45%), medium (32%) and short (18%) seeded-type accessions (length: width ratio 2.5

to 3.0). Cluster II included the long seeded-type Chilean cultivars Brillante-INIA, Cuarzo-INIA, Diamante-INIA, Zafiro-INIA and Buli-INIA, as well as several introduced cultivars, such as Inca, Guara, Fanny, Ranballi, Hispagan, Susan, Karolina, Euro, Alimaniao, IRRi Li-Jian-X-H, Arroz Negro, H404, and Sha-Tiao-Tsao.

At the bottom of the dendrogram, Cluster II included only six genotypes. It was composed mainly by extra large (50%) and long (33%) seeded-type (length:width ratio over 3.0). Most of these accessions corresponded to *aromatic* and scented rice type coming from India (4), Madagascar (1), and China (1). There was one Chilean accession (Quila261601) coming from a cross between 'Sugandh' and 'Ámbar-INIA'.

The principal coordinate analysis that revealed the distribution of the level of genetic diversity in the sample of rice germplasm evaluated showed three main Groups (Groups 1, 2 and 3), similar as those detected by the UPGMA tree, previously. These clusters were specifically

distributed along C1 and C2 in the PCoA plot (Figure 3). In this plot, Group 1 was mainly concentrated in quadrant 2, about 2/3 of the Group 2 was located in quadrant 1, and about 2/3 of Group 3 was located in quadrant 4. Groups 2 and 3, showed intermixing genotypes in quadrant 3. Also, Groups 1 and 2 showed some intermixing between each other. On the overall, about 70% of the total variation was explained by the first three principal coordinates, which indicate that the three clusters from the temperate *japonica* and non *japonica* rice germplasm evaluated are diverse one

to each other. Population structure of the 249 rice accessions was analyzed by Bayesian based approach. The estimated membership fractions of the 249 accessions for different k values, ranged from 2 to 5 (Figure 4). The log likelihood revealed by the structure showed that the optimum k value was 3 ($K = 3$). Similarly, the best ΔK was 3 (Figure 5), which indicated that the entire population evaluated could be clustered into three groups. In summary, at the $K = 3$, the classification based on the structure analysis showed three major groups with a high admixture among the genotypes.

Figure 3. Principal coordinate analysis showing spatial distribution of 249 temperate *japonica* rice accessions.

Figure 4. Variation of 249 rice accessions analyzed by 30 SSR markers (at $K = 2, 3, 4$ and 5) for temperate *japonica* rice germplasm. Membership coefficients (y-axis) within the clusters were determined based on 10 000 iterations using the STRUCTURE program. Bar lengths represent the membership probability of accessions belonging to different groups.

Figure 5. Temperate *japonica* rice population structure of the 249 accessions at $K = 3$ and graph of estimated membership fraction at $K = 3$. The maximum K value was determined by structure harvest, which determined that the population can be grouped into three subgroups.

DISCUSSION

This work is the first comprehensive genotypic analysis of a representative number (249) of the temperate *japonica* germplasm adapted to Chilean conditions, combined with a large number of *loci* (183). Up until now, only a few genetic diversity studies have been carried out on a very small number of genotypes, mainly standard varieties, and using different type of molecular markers such as RAPD (Hinrichsen et al., 1996), AFLPs (Aguirre et al., 2005), and SSR (Becerra et al., 2015).

In this study, genetic diversity analysis revealed a mean for allele number (6.1), major allele frequency

(0.61), minor allele frequency (0.028), number of genotypes (7.03), genetic diversity of 0.52, and PIC value of 0.47. These values are lower than those previously reported when considering the whole genetic structure of rice, that is, *indica*, *aus*, *aromatic*, temperate and *tropical japonica* (Garris et al., 2005; Liakat-Ali et al., 2011; Jamil et al., 2013).

The PIC value for this study ranged from 0.19 to 0.70 with a mean of 0.47 across 30 *loci*. In comparison, Xu et al. (2004) reported a mean PIC value of 0.74, ranging from 0.17 to 0.92, in the world rice collection and a mean PIC value of 0.50, ranging from 0.02 to 0.88, in the US collection. Agrama and Eizenga (2008) reported that wild relatives (*Oryza* spp.), represented by 10 different species, had the highest PIC value (0.78), while the US cultivars had the lowest value (0.39). Another genetic study carried out by Garris et al. (2005) indicated that *indica* and *tropical japonica* groups contained a higher percentage of polymorphic *loci* (99%) and had means of 7.26 and 6.09 alleles per *locus*, respectively. In the same study, the temperate *japonica* group had lower genetic diversity compared with the other rice types with 91% polymorphic *loci* and 4.9 alleles per *locus*. The general mean PIC value across cultivars was 0.67, but the PIC value of the temperate

japonica was only 0.37; PIC value similar as the mean PIC value (0.47) detected in this study.

Gene diversity values in this study had a mean of 0.47 ranging from 0.047 to 0.76, while the US accessions had a mean gene diversity of 0.43 ranging from 0.03 to 0.86 for a single marker. Jin et al. (2010) reported total alleles (390), mean number of alleles (3.9), gene diversity (0.47), and PIC value (0.42) in a collection of 416 accessions originating mostly from China and using 100 SSRs.

The allele numbers of the 249 accessions, analyzed by 30 SSR, generated a total of 183 with an overall mean of 6.1 alleles per *locus* ranging from 2 to 14. Lu et al. (2005) reported 870 alleles that were detected in a sample of 145 US rice collections using 169 SSRs with a mean allele per *locus* of 5.15, which ranged from 2 to 21, PIC value with a range from 0.028 to 0.881 and mean of 0.46, which was similar to the mean gene diversity of the total sample. Mean heterozygosity of the total sample was 3.1%.

A study of a population structure in a core collection of 150 varieties detected 1063 alleles (Zhang et al., 2011). Alleles ranged from 2 to 12 per *locus* with a mean of 3.88 alleles per *locus*, lower than the Chilean values. Mean PIC value was 0.48. Mean alleles per *locus* for *indica* and *japonica* were 3.71 and 3.26, respectively. Gene diversity means for the entire population, *indica* and *japonica* were 0.54, 0.48, and 0.45, respectively. Yan et al. (2010) studied a USDA world rice collection of 1794 accessions from 112 countries in 14 geographic regions, which reported a major allele frequency of 0.52, mean alleles per *locus* of 7.8, gene diversity of 0.61, and PIC value of 0.57.

The observed differences in diversity among rice populations suggest differences in demographic history. Sequence information suggests that *indica* and temperate *japonica* diverged 440 000 years ago (Yamanaka et al., 2004). On the other hand, temperate *japonica*, which has lower genetic diversity and a close genetic relationship with *tropical japonica* (Ni et al., 2002), was derived from the *tropical japonica* group (Garris et al., 2005). Given this scenario, temperate *japonica* accessions need to broaden their genetic diversity primarily from their close relative *tropical japonica* as well as from other groups.

Temperate *japonica* rice is mainly distributed in the cooler regions of East Asia, Central Asia, Europe, North America, and South America. Despite its wide distribution, the relatively low genetic diversity can be attributed to its origin from a narrow gene pool marked by a more severe domestication bottleneck than *indica* or *aus* (Zhu et al., 2007). *Japonica* rice cultivars has a mean PIC value of 0.65 in Yunnan (China) considered its center of diversification (Zeng et al., 2007), higher compared to the mean PIC value (0.47) obtained in this study.

Although the pedigree data available is low, we expected a low genetic diversity of the Chilean RBP due to the high constraint of cold tolerance, photoperiod requirement and seed type. SSR markers allowed to demonstrate that genetic diversity exist, but definitively, the genetic base of the temperate *japonica* rice germplasm used in Chile needs to

be broad in order to create new recombinants to fulfill its requirements.

Population structure

The UPGMA analyses using genetic distance data clustered the 249 genotypes into three main clusters. Cluster I grouped, 243 temperate *japonica* out of 249 total accessions and Cluster II included only six as non *japonica* type. The 243 temperate *japonica* accessions mostly represent breeding materials, commercial and old Chilean cultivars, and foreign cultivars. The genetic breeding material came mainly from INIA's Rice Breeding Program and other rice breeding programs such as Centro Internacional de Agricultura Tropical (CIAT, Colombia), International Rice Research Institute (IRRI, Philippines), and other rice programs.

The subgroup I in Cluster I grouped short and medium grain accessions, along the most representatives short grain types, 'Oro', and two commercial 'Quella-INIA' and 'Ámbar-INIA'. The subgroup II within the Cluster I, which included long and medium type of grain, along with the Chilean commercial cultivars being used today: 'Zafiro-INIA', 'Cuarzo-INIA' among others. The analysis of both subgroups showed most of the INIA cultivars together indicating a high level of genetic relatedness. For example, 'Diamante-INIA' and 'Zafiro-INIA' have a genetic similarity coefficient of 96%. Cluster II contained five accessions Basmati (*aromatic*), Sugandh-2, Sugandh-3 (both scented rice), Chu Xiang, PRA557 and Quila261601. Quila261601 is a Chilean genotype resulting from the hybridization of 'Ámbar-INIA' × Sugandh-2. This observation agreed with Kumari et al. (2011) and Chuang et al. (2011) that indicated that microsatellites were able to differentiate medium/narrow seeded-type Indian varieties, and domestic from foreign rice varieties in Taiwan, respectively. It has already been reported that some SSRs are associated with regions of DNA that determine the grain type (Huang et al., 2013). Additionally, grain size is one of the important criteria for determining the genetic structure of the rice germplasm (Courtois et al., 2012).

However, grain size did not have a clear relationship between the observed groups within the dendrogram, the major groups showed a mix of the three types of grains. Historically, the first Chilean cultivars were short-seeded type, but since the 80's there was a big change on Chilean consumer's preference, and the short short-seeded type was changed to a long-wide seeded type. It is known that long-seeded type are generally produced in African, Central and South American, North American, and Caribbean and most of those genotypes are classified as *tropical japonica*. On the other hand, East Asian, Central Asian, and South Asian produces shorter grains with about half of the East Asian and more than half of the Central Asian accessions classified as temperate *japonica*. Then, the Chilean challenge is to develop a long-width seed type temperate germplasm with good cold tolerance at vegetative and reproductive stages.

Although, the genetic distance based approach is powerful, easy to use, and has been widely reported on genetic studies. This kind of analysis may have a potential problem, because the number of identified groups is based on an arbitrary cut off which depends on the researcher's judgment. In this study, the genetic structure given by the dendrogram, based on the genetic distance approach, is also supported by a very important morphological and commercial trait and the majority of the rice accessions were grouped based on their seed size.

Additionally a PCoA and model-based method, such as Structure, also suggested the existence of three major groups, corresponding to temperate *japonica* and non *japonica* types. A different intensity of intermixing populations was observed with the PCoA plot. A 70% variation was observed with the first three principal coordinates, which indicated that rice accessions were diverse from one to each other.

Structure uses a Bayesian clustering approach in which each group or population is based on the likelihood for each number of groups (K). This approach enables to choose the number of groups with the highest log likelihood (Lu et al., 2005). The Population structure analysis of different rice diversity panels has indicated different numbers of subgroups, from 2 to 8 (Garris et al., 2005; Liakat-Ali et al., 2011; Das et al., 2013). The 249 accessions of this study were organized by the structure analysis into three main groups and revealed a fairly consistent genetic relationship with the dendrogram and the PCoA. The Temperate *japonica* accessions can be further subdivided into three subpopulations where the long and short Chilean varieties were grouped into different clusters. The three populations, showed different level of admixture, admixture probably due to the previous breeding work through years.

CONCLUSIONS

The polymorphism level detected by simple sequences repeats (SSR) is generally medium to low among INIA rice (*Oryza sativa* L.) germplasm. This is supported by the diversity parameters, such as the number of alleles (6.1) and polymorphism index content (PIC, 0.47). Given this situation, it is important to continue the introduction of germplasm from other temperate and subtropical regions to increase genetic diversity of the Rice Breeding Program.

Genetic clustering of the 249 rice accessions using SSR with Jaccard coefficient determined the separation of *japonica* and non *japonica* rice. This clustering was also related to the seeded-size type; thus, genotypes were clustered according to grain length/width: short/wide, long/wide, and long/narrow ratio.

Population structure and Principal Coordinates Analysis determined three groups, and indicated that the temperate *japonica* germplasm adapted to Chile has a high level of admixture.

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