# 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 grain 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.

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## 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 (35º51' 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

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° | Accesion | Origin | Subspecies | Grain type | Structure |
|---|---|---|---|---|---|
| 1 | Oro | Chile | *japonica* | Short | Group3 |
| 2 | Ambar-INIA | Chile | *japonica* | Short | Group1 |
| 3 | Quella-INIA | Chile | *japonica* | Short | Group3 |
| 4 | Brillante-INIA | Chile | *japonica* | Long | Group2 |
| 5 | Buli-INIA | Chile | *japonica* | Long | Group2 |
| 6 | Diamante-INIA | Chile | *japonica* | Long | Group2 |
| 7 | Cuarzo-INIA | Chile | *japonica* | Long | Group2 |
| 8 | Zafiro-INIA | Chile | *japonica* | Long | Group2 |
| 9 | CINIA 609 | Chile | *japonica* | Long | Group2 |
| 10 | Quila 157302 | Chile | *japonica* | Short | Group2 |
| 11 | Quila 154601 | Chile | *japonica* | Medium | Group3 |
| 12 | Quila 154804 | Chile | *japonica* | Medium | Group3 |
| 13 | Quila 156603 | Chile | *japonica* | Medium | Group3 |
| 14 | Quila 159005 | Chile | *japonica* | Short | Group2 |
| 15 | Quila 173201 | Chile | *japonica* | Medium | Group3 |
| 16 | Quila 185007 | Chile | *japonica* | Medium | Group3 |
| 17 | Quila 213801 | Chile | *japonica* | Medium | Group3 |
| 18 | Quila 216305 | Chile | *japonica* | Long | Group3 |
| 19 | Quila 221801 | Chile | *japonica* | Long | Group3 |
| 20 | Quila 225001 | Chile | *japonica* | Medium | Group3 |
| 21 | Quila 225101 | Chile | *japonica* | Medium | Group3 |
| 22 | Quila 225103 | Chile | *japonica* | Long | Group3 |
| 23 | Quila 228603 | Chile | *japonica* | Long | Group3 |
| 24 | Quila 230513 | Chile | *japonica* | Extra Large | Group1 |
| 25 | Quila 230601 | Chile | *japonica* | Long | Group3 |
| 26 | Quila 230602 | Chile | *japonica* | Long | Group3 |
| 27 | Quila 230603 | Chile | *japonica* | Long | Group3 |
| 28 | Quila 231902 | Chile | *japonica* | Medium | Group3 |
| 29 | Quila 233008 | Chile | *japonica* | Medium | Group3 |
| 30 | Quila 234801 | Chile | *japonica* | Long | Group1 |
| 31 | Quila 235207 | Chile | *japonica* | Long | Group3 |
| 32 | Quila 235501 | Chile | *japonica* | Long | Group2 |
| 33 | Quila 237908 | Chile | *japonica* | Long | Group3 |
| 34 | Platino-INIA | Chile | *japonica* | Short | Group1 |
| 35 | Quila 242104 | Chile | *japonica* | Short | Group1 |
| 36 | Corea 2 | Corea | *japonica* | Short | Group3 |
| 37 | Guadiamar | España | *japonica* | Short | Group1 |
| 38 | Guara | España | *japonica* | Medium | Group3 |
| 39 | Hispagran | U.S.A | *japonica* | Medium | Group3 |
| 40 | Susan | España | *japonica* | Medium | Group3 |
| 41 | Euro | Europa | *japonica* | Medium | Group3 |
| 42 | Ranballi | Bulgaria | *japonica* | Short | Group1 |
| 43 | Karolina | Hungría | *japonica* | Long | Group3 |
| 44 | Basmati C621 | India | *aromatic* | Long | Group2 |
| 45 | Basmati | India | *aromatic* | Long | Group3 |
| 46 | Sugandh-2 | India | *aromatic* | Extra Large | Group3 |
| 47 | Sugandh-3 | India | *aromatic* | Extra Large | Group3 |
| 48 | Chu Xiang | China | *japonica* | Medium | Group3 |
| 49 | Arroz Negro | Brasil | *japonica* | Medium | Group3 |
| 50 | IRRI-Li Jian-X-H | China | *japonica* | Extra Large | Group3 |
| 51 | IRRI-Yuhkara | Japón | *japonica* | Extra Large | Group3 |
| 52 | Quila 213007 | Chile | *japonica* | Medium | Group3 |
| 53 | Rquila 17 | Chile | *japonica* | Long | Group2 |
| 54 | Quila 242101 | Chile | *japonica* | Short | Group3 |
| 55 | Quila 222704 | Chile | *japonica* | Long | Group3 |
| 56 | Quila 208902 | Chile | *japonica* | Long | Group2 |
| 57 | Quila 216501 | Chile | *japonica* | Long | Group2 |
| 58 | Quila 194603 | Chile | *japonica* | Long | Group2 |
| 59 | Quila 200112 | Chile | *japonica* | Medium | Group3 |
| 60 | Quila 242610 | Chile | *japonica* | Short | Group1 |
| 61 | Quila 242207 | Chile | *japonica* | Long | Group1 |
| 62 | INIAG 70 | Chile | *japonica* | Long | Group2 |
| 63 | Quila 242420 | Chile | *japonica* | Medium | Group1 |
| 64 | Quila 241319 | Chile | *japonica* | Medium | Group1 |
| 65 | Quila 242616 | Chile | *japonica* | Short | Group1 |
| 66 | Quila 194602 | Chile | *japonica* | Long | Group2 |
| 67 | Quila 222703 | Chile | *japonica* | Long | Group3 |
| 68 | Quila 240101 | Chile | *japonica* | Long | Group2 |
| 69 | Quila INIAG 152 | Chile | *japonica* | Long | Group2 |
| 70 | Quila 224802 | Chile | *japonica* | Long | Group2 |

Continuation Table 1.

| Nº | Accesion | Origin | Subspecies | Grain type | Structure |
|---|---|---|---|---|---|
| 71 | INIAG 144 | Chile | *japonica* | Long | Group2 |
| 72 | Quila 216202 | Chile | *japonica* | Long | Group2 |
| 73 | INIAG 115 | Chile | *japonica* | Long | Group2 |
| 74 | Quila 242703 | Chile | *japonica* | Long | Group2 |
| 75 | Rquila 363 | Chile | *japonica* | Long | Group2 |
| 76 | INIAG 99 | Chile | *japonica* | Long | Group2 |
| 77 | Rquila 205 | Chile | *japonica* | Long | Group2 |
| 78 | INIAG 169 | Chile | *japonica* | Long | Group2 |
| 79 | INIAG 165 | Chile | *japonica* | Long | Group2 |
| 80 | Quila 249006 | Chile | *japonica* | Long | Group1 |
| 81 | Quila 249104 | Chile | *japonica* | Long | Group2 |
| 82 | Quila 249203 | Chile | *japonica* | Long | Group2 |
| 83 | Quila 223105 | Chile | *japonica* | Extra Large | Group3 |
| 84 | Quila 231701 | Chile | *japonica* | Extra Large | Group3 |
| 85 | Quila 242203 | Chile | *japonica* | Medium | Group2 |
| 86 | Quila 222204 | Chile | *japonica* | Long | Group2 |
| 87 | Quila 242002 | Chile | *japonica* | Long | Group2 |
| 88 | Rquila 28 | Chile | *japonica* | Long | Group3 |
| 89 | INIAG 79 | Chile | *japonica* | Long | Group2 |
| 90 | Quila 256603 | Chile | *japonica* | Long | Group2 |
| 91 | Quila 256001 | Chile | *japonica* | Long | Group2 |
| 92 | Quila 256002 | Chile | *japonica* | Long | Group2 |
| 93 | Quila 256602 | Chile | *japonica* | Long | Group2 |
| 94 | Quila 251702 | Chile | *japonica* | Long | Group3 |
| 95 | Quila 256601 | Chile | *japonica* | Long | Group2 |
| 96 | Quila 256501 | Chile | *japonica* | Long | Group2 |
| 97 | Quila 252801 | Chile | *japonica* | Long | Group2 |
| 98 | Quila 254101 | Chile | *japonica* | Long | Group2 |
| 99 | Quila 244013 | Chile | *japonica* | Long | Group2 |
| 100 | Quila 241801 | Chile | *japonica* | Medium | Group1 |
| 101 | Quila 241612 | Chile | *japonica* | Medium | Group2 |
| 102 | Quila 242206 | Chile | *japonica* | Medium | Group1 |
| 103 | Quila 243008 | Chile | *japonica* | Short | Group1 |
| 104 | Quila 242010 | Chile | *japonica* | Short | Group2 |
| 105 | Quila 223202 | Chile | *japonica* | Medium | Group3 |
| 106 | Quila 241610 | Chile | *japonica* | Medium | Group2 |
| 107 | Quila 241703 | Chile | *japonica* | Short | Group2 |
| 108 | Quila 225105 | Chile | *japonica* | Medium | Group3 |
| 109 | Quila 242011 | Chile | *japonica* | Medium | Group2 |
| 110 | RQuila 356 | Chile | *japonica* | Medium | Group3 |
| 111 | Quila 251301 | Chile | *japonica* | Extra Large | Group3 |
| 112 | Quila 254102 | Chile | *japonica* | Extra Large | Group3 |
| 113 | Quila 254701 | Chile | *japonica* | Extra Large | Group3 |
| 114 | Quila 253701 | Chile | *japonica* | Extra Large | Group2 |
| 115 | Quila 260312 | Chile | *japonica* | Long | Group3 |
| 116 | Quila 260404 | Chile | *japonica* | Long | Group3 |
| 117 | Quila 242003 | Chile | *japonica* | Medium | Group2 |
| 118 | Quila 242701 | Chile | *japonica* | Medium | Group2 |
| 119 | Quila 242006 | Chile | *japonica* | Medium | Group2 |
| 120 | Quila 242504 | Chile | *japonica* | Medium | Group2 |
| 121 | Quila 242115 | Chile | *japonica* | Short | Group1 |
| 122 | Quila 242007 | Chile | *japonica* | Medium | Group2 |
| 123 | Quila 242802 | Chile | *japonica* | Medium | Group2 |
| 124 | Quila 240103 | Chile | *japonica* | Medium | Group3 |
| 125 | Quila 240204 | Chile | *japonica* | Medium | Group1 |
| 126 | Quila 240208 | Chile | *japonica* | Medium | Group1 |
| 127 | Quila 241606 | Chile | *japonica* | Medium | Group1 |
| 128 | Quila 249301 | Chile | *japonica* | Short | Group1 |
| 129 | Quila 243010 | Chile | *japonica* | Medium | Group3 |
| 130 | Quila 241305 | Chile | *japonica* | Medium | Group1 |
| 131 | Quila 241313 | Chile | *japonica* | Short | Group1 |
| 132 | Quila 225102 | Chile | *japonica* | Medium | Group3 |
| 133 | Quila 242415 | Chile | *japonica* | Medium | Group2 |
| 134 | Quila 253003 | Chile | *japonica* | Short | Group1 |
| 135 | Quila 249002 | Chile | *japonica* | Medium | Group3 |
| 136 | Quila 240201 | Chile | *japonica* | Short | Group1 |
| 137 | Quila 241315 | Chile | *japonica* | Medium | Group1 |
| 138 | Quila 242012 | Chile | *japonica* | Short | Group1 |
| 139 | INIAG 27 | Chile | *japonica* | Short | Group1 |
| 140 | Quila 241605 | Chile | *japonica* | Short | Group1 |
| 141 | Quila 242106 | Chile | *japonica* | Short | Group3 |
| 142 | Quila 242108 | Chile | *japonica* | Short | Group1 |
| 143 | Quila 241701 | Chile | *japonica* | Short | Group2 |
| 144 | Quila 241607 | Chile | *japonica* | Short | Group1 |
| 145 | Quila 241321 | Chile | *japonica* | Short | Group1 |
| 146 | Quila 241304 | Chile | *japonica* | Short | Group1 |
| 147 | Quila 249101 | Chile | *japonica* | Short | Group1 |
| 148 | Quila 249103 | Chile | *japonica* | Short | Group1 |
| 149 | Quila 249303 | Chile | *japonica* | Short | Group1 |
| 150 | Quila 249304 | Chile | *japonica* | Short | Group1 |
| 151 | Quila 251303 | Chile | *japonica* | Short | Group3 |
| 152 | Quila 252702 | Chile | *japonica* | Medium | Group3 |
| 153 | Quila 252201 | Chile | *japonica* | Short | Group1 |
| 154 | Quila 242204 | Chile | *japonica* | Short | Group1 |
| 155 | Quila 242609 | Chile | *japonica* | Short | Group1 |
| 156 | Quila 242612 | Chile | *japonica* | Short | Group1 |
| 157 | Quila 242112 | Chile | *japonica* | Short | Group1 |
| 158 | Quila 242613 | Chile | *japonica* | Short | Group1 |
| 159 | Quila 241307 | Chile | *japonica* | Medium | Group1 |
| 160 | Quila 242608 | Chile | *japonica* | Short | Group1 |
| 161 | Quila 242808 | Chile | *japonica* | Short | Group1 |
| 162 | Quila 242121 | Chile | *japonica* | Short | Group1 |
| 163 | Quila 242114 | Chile | *japonica* | Short | Group1 |
| 164 | Quila 256104 | Chile | *japonica* | Short | Group1 |
| 165 | Quila 256103 | Chile | *japonica* | Short | Group1 |
| 166 | Quila 256106 | Chile | *japonica* | Short | Group1 |
| 167 | Quila 256101 | Chile | *japonica* | Short | Group1 |
| 168 | Quila 256701 | Chile | *japonica* | Short | Group3 |
| 169 | Quila 256901 | Chile | *japonica* | Short | Group2 |
| 170 | Quila 256903 | Chile | *japonica* | Short | Group1 |
| 171 | Quila 256902 | Chile | *japonica* | Short | Group2 |
| 172 | Quila 249201 | Chile | *japonica* | Medium | Group1 |
| 173 | Quila 249501 | Chile | *japonica* | Medium | Group3 |
| 174 | Quila 251703 | Chile | *japonica* | Medium | Group2 |
| 175 | Quila 252701 | Chile | *japonica* | Medium | Group3 |
| 176 | Quila 256401 | Chile | *japonica* | Medium | Group3 |
| 177 | Quila 256604 | Chile | *japonica* | Medium | Group2 |
| 178 | Quila 256402 | Chile | *japonica* | Medium | Group2 |
| 179 | Quila 256605 | Chile | *japonica* | Medium | Group2 |
| 180 | Quila 256801 | Chile | *japonica* | Medium | Group2 |
| 181 | Quila 260405 | Chile | *japonica* | Medium | Group3 |
| 182 | Quila 240102 | Chile | *japonica* | Medium | Group3 |
| 183 | Quila 241403 | Chile | *japonica* | Medium | Group1 |
| 184 | Quila 240203 | Chile | *japonica* | Medium | Group1 |
| 185 | Quila 244104 | Chile | *japonica* | Medium | Group1 |
| 186 | Quila 243306 | Chile | *japonica* | Medium | Group1 |
| 187 | Quila 245801 | Chile | *japonica* | Short | Group3 |
| 188 | Quila 243201 | Chile | *japonica* | Short | Group1 |
| 189 | Quila 220001 | Chile | *japonica* | Medium | Group2 |
| 190 | Quila 257501 | Chile | *japonica* | Short | Group1 |
| 191 | Quila 257003 | Chile | *japonica* | Short | Group1 |
| 192 | Quila 251302 | Chile | *japonica* | Short | Group1 |
| 193 | Quila 257502 | Chile | *japonica* | Short | Group1 |
| 194 | Quila 249005 | Chile | *japonica* | Short | Group1 |
| 195 | Quila 249305 | Chile | *japonica* | Short | Group1 |
| 196 | Quila 241402 | Chile | *japonica* | Medium | Group1 |
| 197 | Quila 243901 | Chile | *japonica* | Medium | Group2 |
| 198 | Quila 241608 | Chile | *japonica* | Short | Group2 |
| 199 | Quila 242617 | Chile | *japonica* | Short | Group1 |
| 200 | Quila 243102 | Chile | *japonica* | Short | Group1 |
| 201 | Quila 244012 | Chile | *japonica* | Short | Group1 |
| 202 | Quila 244501 | Chile | *japonica* | Short | Group1 |
| 203 | Quila 243304 | Chile | *japonica* | Short | Group3 |
| 204 | Quila 242118 | Chile | *japonica* | Short | Group3 |

Continuation Table 1.

| Nº | Accesion | Origin | Subspecies | Grain type | Structure |
|---|---|---|---|---|---|
| 205 | Quila 246901 | Chile | *japonica* | Medium | Group3 |
| 206 | Quila 241312 | Chile | *japonica* | Short | Group1 |
| 207 | Quila 242008 | Chile | *japonica* | Short | Group2 |
| 208 | INIAG 220 | Chile | *japonica* | Long | Group2 |
| 209 | Quila 238803 | Chile | *japonica* | Long | Group3 |
| 210 | Quila 238908 | Chile | *japonica* | Long | Group3 |
| 211 | Quila 242004 | Chile | *japonica* | Long | Group2 |
| 212 | Quila 242005 | Chile | *japonica* | Medium | Group2 |
| 213 | Quila 242205 | Chile | *japonica* | Short | Group1 |
| 214 | Quila 257004 | Chile | *japonica* | Short | Group1 |
| 215 | Quila 257301 | Chile | *japonica* | Short | Group1 |
| 216 | Quila 258301 | Chile | *japonica* | Short | Group1 |
| 217 | Quila 261601 | Chile | *japonica* | Long | Group3 |
| 218 | Quila 261801 | Chile | *japonica* | Long | Group3 |
| 219 | Quila 261803 | Chile | *japonica* | Long | Group3 |
| 220 | Quila 262003 | Chile | *japonica* | Short | Group3 |
| 221 | Quila 262101 | Chile | *japonica* | Long | Group2 |
| 222 | Quila 262102 | Chile | *japonica* | Long | Group2 |
| 223 | Quila 262103 | Chile | *japonica* | Extra Large | Group3 |
| 224 | Quila 262104 | Chile | *japonica* | Extra Large | Group3 |
| 225 | Quila 262301 | Chile | *japonica* | Short | Group3 |
| 226 | Quila 262401 | Chile | *japonica* | Long | Group3 |
| 227 | Quila 262602 | Chile | *japonica* | Short | Group3 |
| 228 | Quila 262802 | Chile | *japonica* | Long | Group3 |
| 229 | Quila 262803 | Chile | *japonica* | Long | Group3 |
| 230 | Quila 263102 | Chile | *japonica* | Long | Group3 |
| 231 | Quila 263304 | Chile | *japonica* | Long | Group3 |
| 232 | Quila 263901 | Chile | *japonica* | Short | Group3 |
| 233 | Quila 264001 | Chile | *japonica* | Long | Group2 |
| 234 | Quila 264601 | Chile | *japonica* | Short | Group3 |
| 235 | Quila 265105 | Chile | *japonica* | Medium | Group2 |
| 236 | Quila 265602 | Chile | *japonica* | Long | Group3 |
| 237 | Quila 266001 | Chile | *japonica* | Short | Group3 |
| 238 | Quila 266002 | Chile | *japonica* | Short | Group2 |
| 239 | Quila 266405 | Chile | *japonica* | Short | Group3 |
| 240 | Quila 266502 | Chile | *japonica* | Long | Group3 |
| 241 | Inca | Colombia | *japonica* | Long | Group2 |
| 242 | Sha-Tiao-Tsao | China | *japonica* | Medium | Group3 |
| 243 | Alinamo. C. | Colombia | *japonica* | Long | Group3 |
| 244 | CT6742-12-CA-32 | Colombia | *japonica* | Extra Large | Group2 |
| 245 | H404 | Hungría | *japonica* | Medium | Group3 |
| 246 | Fanny | Francia | *japonica* | Medium | Group3 |
| 247 | Quila 154907 | Chile | *japonica* | Extra Large | Group2 |
| 248 | CT6750-9-2-4-2-M-M-3 | Colombia | *japonica* | Long | Group2 |
| 249 | PRA557 | Madagascar | *japonica* | 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*Hind*III 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 ηg $\mu L^{-1}$ 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 $\mu$L total volume made up of 0.1 µM of each primer, 1 unit of *Taq* DNA polymerase, 0.2 µM of each dNTP, 10 mM Tris-HCl pH 7.2, 50 mM KCl, 1.5 mM $MgCl_2$, DMSO (50%), and 10 ηg 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 $\mu$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 (ºC) |
|---|---|---|---|---|
| RM1164 | 3 | CGTTTCTCCGAGAAAAGTCG | CAAGGTGGTCGTTGAGGC | 55 |
| RM8068 | 1 | AAACCTCTCGCTGTAATTAG | TGAACATTTATTGATATGGTAAA | 57 |
| RM413 | 5 | GGCGATTCTTGGATGAAGAG | TCCCCACCAATCTTGTCTTC | 53 |
| RM286 | 11 | GGCTTCATCTTTGGCGAC | CCGGATTCACGAGATAAACTC | 55 |
| RM10 | 7 | TTGTCAAGAGGAGGCATCG | CAGAATGGGAAATGGGTCC | 55 |
| RM502 | 8 | GCGATCGATGGCTACGAC | ACAACCCAACAAGAAGGACG | 55 |
| RM21 | 11 | ACAGTATTCCGTAGGCACGG | GCTCCATGAGGGTGGTAGAG | 55 |
| OSR28 | 9 | AGCAGCTATAGCTTAGCTGG | ACTGCACATGAGCAGAGACA | 55 |
| RM44 | 8 | ACGGGCAATCCGAACAACC | TCGGGAAAACCTACCCTACC | 53 |
| RM276 | 6 | CTCAACGTTGACACCTCGTG | TCCTCCATCGAGCAGTATCA | 55 |
| RM259 | 1 | TGGAGTTTGAGAGGAGGG | CTTGTTGCATGGTGCCATGT | 55 |
| RM1230 | 3 | GGGTGGTGTGAGCTTTTCTC | TTCCACTTCGACAACCCTTC | 55 |
| RM482 | 2 | TCTGAAAGCCTGACTCATCG | GTCAATTGCAGTGCCCTTTC | 55 |
| RM537 | 4 | CCGTCCCTCTCTCTCCTTTC | ACAGGGAAACCATCCTCCTC | 55 |
| RM547 | 8 | TAGGTTGGCAGACCTTTTCG | GTCAAGATCATCCTCGTAGCG | 55 |
| RM561 | 2 | GAGCTGTTTTGGACTACGGC | GAGTAGCTTTCTCCCACCCC | 55 |
| RM560 | 7 | GCAGGAGGAACAGAATCAGC | AGCCCGTGATACGGTGATAG | 55 |
| RM1261 | 12 | GTCCATGCCCAAGACACAAC | GTTACATCATGGGTGACCCC | 55 |
| RM241 | 4 | GAGCCAAATAAGATCGCTGA | TGCAAGCAGCAGATTTAGTG | 55 |
| RM426 | 3 | ATGAGATGAGTTCAAGGCCC | AACTCTGTACCTCCATCGCC | 55 |
| RM17 | - | TGCCCTGTTATTTTCTTCTCTC | GGTGATCCTTTCCCATTTCA | 55 |
| RM243 | - | GATCTGCAGACTGCAGTTGC | AGCTGCAACGATGTTGTCC | 55 |
| RM406 | 2 | GAGGGAGAAAGGTGGACATG | TGTGCTCCTTGGGAAGAAAG | 55 |
| RM447 | 8 | CGGTGTGTAAAACTCCGAAGCACC | TGCCGTGGCTCATTAGTGGTC | 55 |
| RM509 | 5 | TAGTGAGGGAGTGGAAACGG | ATCGTCCCCACAATCTCATC | 55 |
| RM510 | 6 | AACCGGATTAGTTTCTCGCC | TGAGGACGACGAGCAGATTC | 55 |
| RM525 | 2 | GGCCCGTCCAAGAAATATTG | CGGTGAGACAGAATCCTTACG | 55 |
| RM555 | 2 | TTGGATCAGCCAAAGGAGAC | CAGCATTGTGGCATGGATAC | 55 |
| RM583 | 1 | AGATCCATCCCTGTGGAGAG | GCGAACTCGCGTTGTAATC | 55 |
| RM5463 | 6 | ACCCTTGCAGACAACGTACC | 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 COPH 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

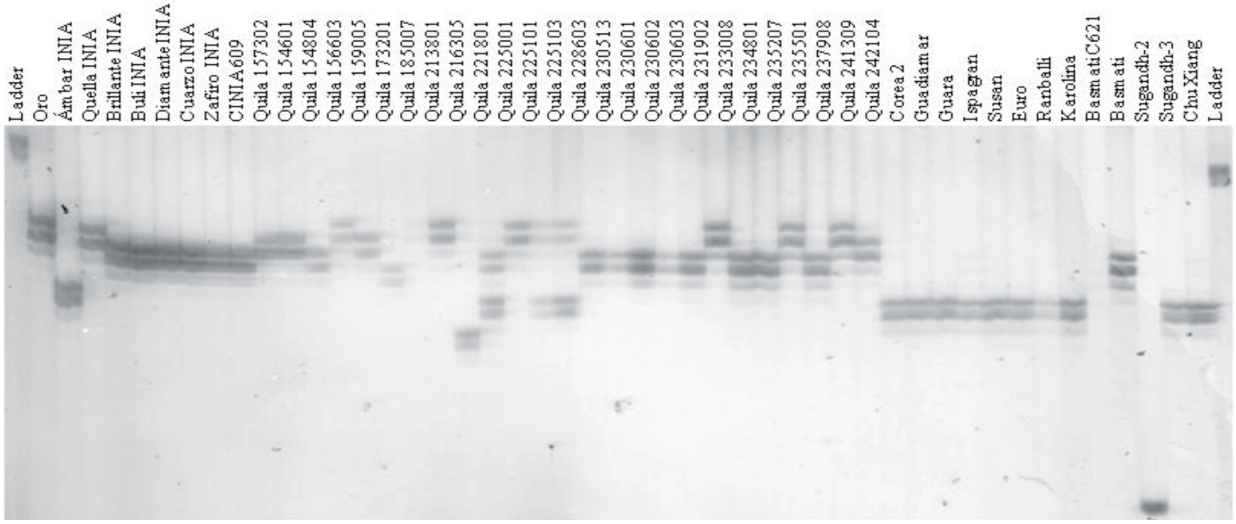
A total of 30 polymorphic microsatellites were selected and used to evaluate the total of 249 rice accessions (Table 2). The total number of alleles scored across the 249 genotypes was 183 with an overall mean of 6.1 alleles per *locus* ranging from 2 to 14 (Table 3, Figure 1). The most polymorphic *loci* 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 (He) 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.**

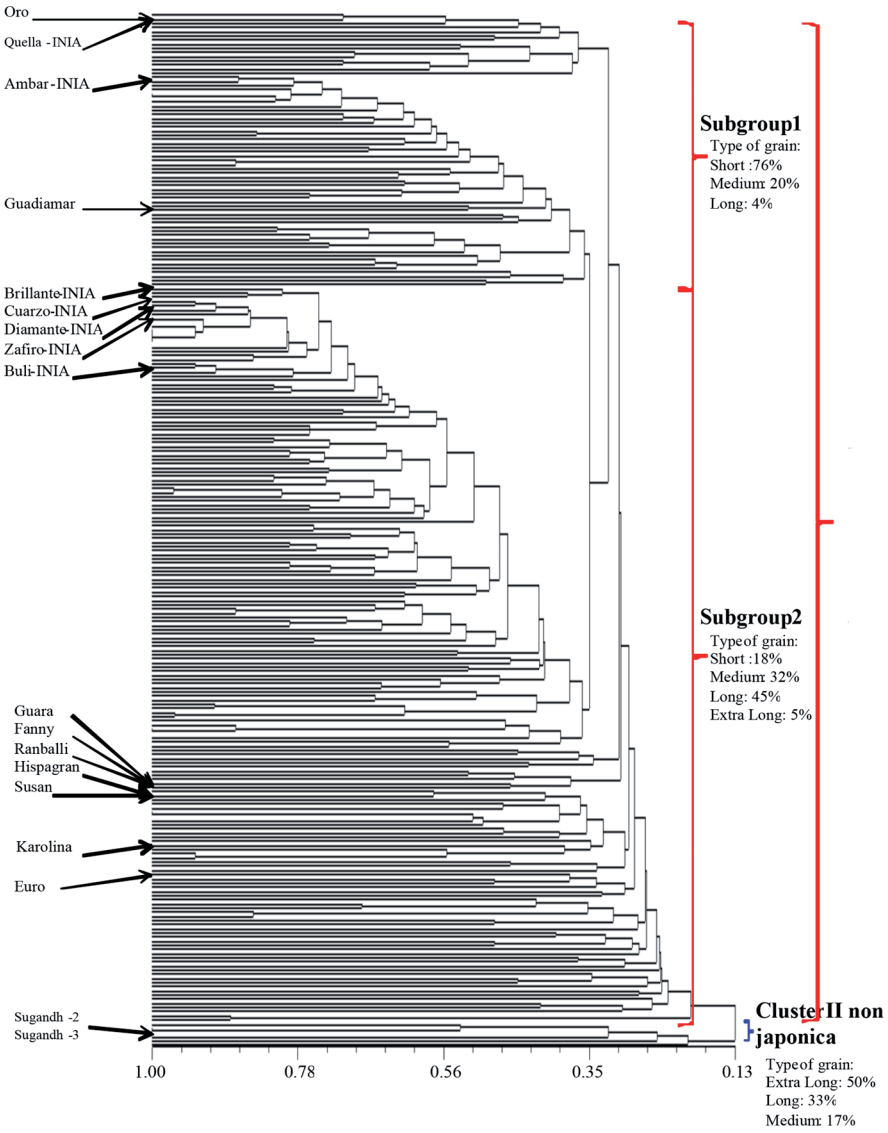



detected by RM44 and RM241. The mean heterozygosity was very low (0.01) and the highest value was detected by RM560 (0.05) and several *loci* did not detect any level of heterozygosity. The PIC value for the SSR *loci* ranged from 0.19 (RM1230) to 0.70 (RM44) with a mean of 0.47 across of the 30 *loci*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

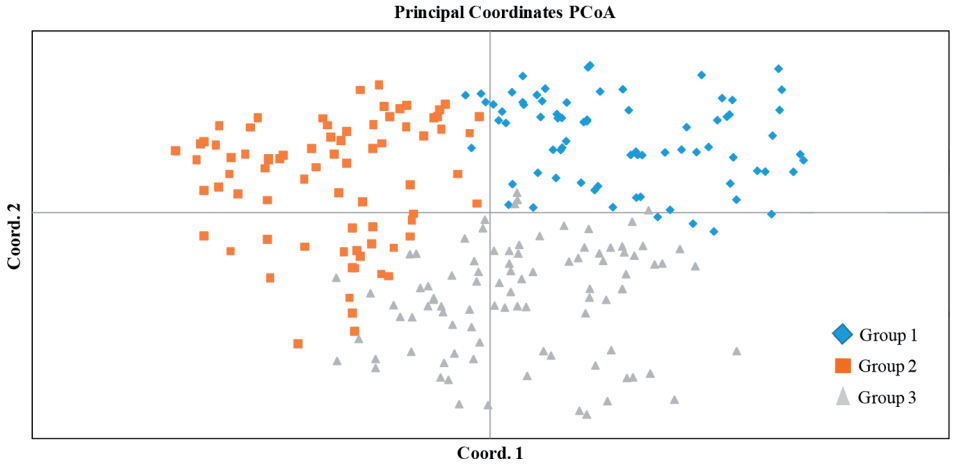



**Figure 4. Variation of 249 rice accessions analyzed by 30 SSR markers (at K = 2, 3, 4 and 5) for temperate *japonica* rice germplasm. Membership coefficients (y-axis) within the clusters were determined based on 10 000 iterations using the STRUCTURE program. Bar lengths represent the membership probability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

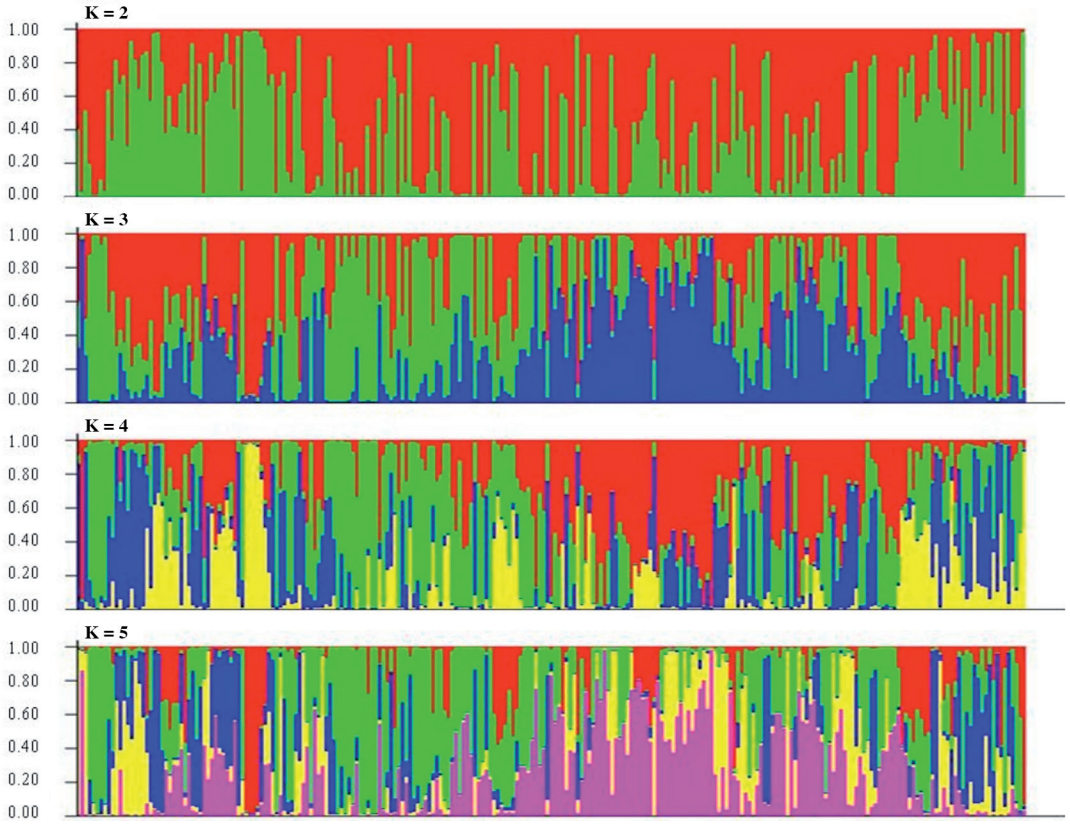
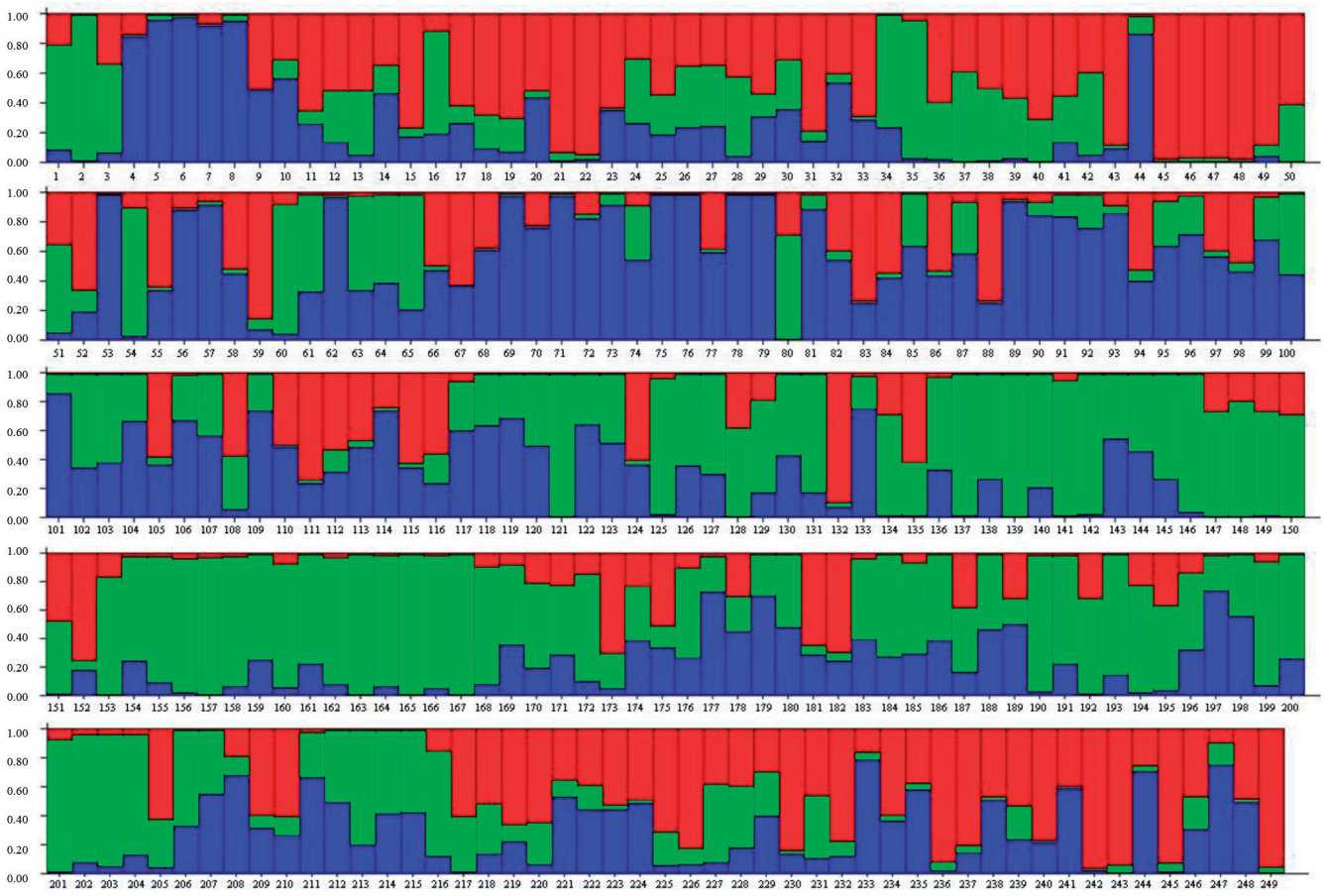
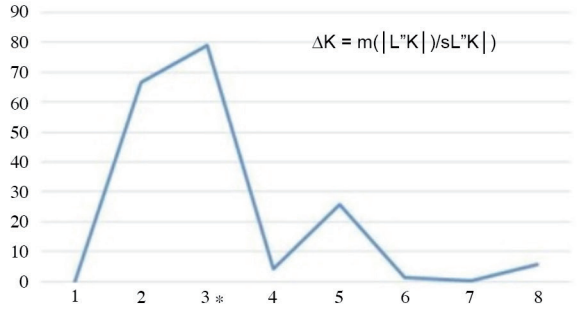


**Figure 5. Temperate *japonica* rice population structure of the 249 accessions at K = 3 and graph of estimated membership fraction at K = 3. The maximum K value was determined by structure harvest, which determined that the population can be grouped into three subgroups.**






## 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 accesions 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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