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RESEARCH ARTICLE

Molecular profiling of rice (*Oryza sativa* L.) genotypes using trait-based SNP markers linked to yield under drought condition

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Abstract

Drought is one of the major abiotic stress factors affecting the growth and production of rice globally and it can cause an estimated yield loss up to 90% in rice. With the increasing population around the globe, a comprehensive approach for mitigating drought stress should be taken to develop drought-tolerant rice varieties to meet future food demands. One hundred and eighty-two rice genotypes were evaluated for six critical gene/QTLs associated with drought tolerance using SNP marker data. The results revealed that 172 genotypes carry at least one gene/QTL for drought tolerance. The six gene/QTLs, namely, *DTY1.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2*, *qDTY4.1* and *qDTY12.1*were found in 103, 26, 90, 25, 27 and 45 genotypes corresponding to 57, 49, 14, 14, 15 and 25% of the total screened genotypes, respectively. Remarkably, five genotypes (RL-32, RL-105, RL-110, RL-142 and RL-158) possessed a unique combination three major genes/QTLs and three genotypes (RL-21, RL-41 and RL-188) possessed a unique combination four major gene/QTLs for drought tolerance. Furthermore, both cluster and populationn structure analyses revealed the distribution of the genotypes into two major clusters. The genotypes carrying valuable gene/QTLs either in single or combination, hold immense potential for deployment in drought tolerance rice breeding programs.

Keywords: Abiotic stress, drought tolerance, rice, SNP and QTLs

Introduction

Rice is primarily grown and consumed to a larger extent in Asia. Rice is a majorly grown cereal crop in India and across the world and feeds more than 50% of the population across the globe. As the global population continues to increase, it becomes imperative to take proactive measures to simultaneously increase rice production and to meet future demands(Raju et al. 2023). Rice is a semi-aquatic crop, which means it requires high water for its cultivation, making it particularly more prone to drought stress (Arsode et al.2022). Rice production is limited by major biotic stresses (fungus, insect pests and bacteria) and abiotic stresses (drought and salinity). During the life cycle of rice crop, the reproductive stage is more vulnerable to drought compared to the vegetative stage, which can delay or inhibit the flowering, grain development, and ultimately yield, involving the integration of several biochemical, physiological and genetic processes. Kumar et al. (2008) reported drought stress's devastating effects on rice at panicle initiation and flowering (Zhang et al. 2018; Raju et al. 2023) revealed that drought causes an estimated yield loss up to 21% under mild drought, 51% under moderate drought and 90.6% in extreme cases, depending on the growth stage, variety and period of the stress. Therefore, drought tolerance in rice is of significant importance, especially in regions prone to water scarcity and the challenges arising from the changing climate patterns. Thus, there is an urgent need to address this challenge through a sustained and comprehensive

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approach encompassing plant breeding, biotechnological and agronomical aspects.

The huge rice germplasm resource offers a promising avenue for tackling drought stress due to its abundant genetic diversity. Harnessing this diversity for the development of drought-tolerant rice varieties through systematic breeding approaches is a critical step in ensuring food security in the face of changing climatic conditions. Previous reports have revealed different methods to screen rice genotypes for drought tolerance through conventional approaches and molecular breeding strategies. Sandhu et al. (2017) have discussed the rich and diverse available rice genetic resources like (wild species, mutant genotypes, recombinant inbred lines, near-isogenic lines, double haploids and advanced breeding populations)as an invaluable material in selecting suitable donors and mapping quantitative trait loci (QTLs) for drought conditions.

Several systematic studies have been carried out, involving various mapping populations for mapping of major QTLs associated with grain yield under drought conditions and their subsequent application through introgression (Kumar et al. 2014). Shamsudin et al. (2016) has introgressed these QTLs, *qDTY2.2*, *qDTY3.2* and *qDTY12.1*to the Malaysian variety MRQ74 and found these QTLs in 18, 36 and 82% of the selected introgressed lines, respectively. Molecular markers play a key role in screening rice genotypes (Ashfaq et al. 2014; Verma et al. 2019). Presently, DNA/molecular markers have laid the way to screen a large number of accessions for identifying genotypes for the presence of several genes/QTLs governing various traits in rice. Notably, single nucleotide polymorphic (SNP) markers are indeed widely used due to their high-throughput nature, low mutation rates, genome-wide coverage and high frequency (Arif et al. 2019; Ndikuryayo et al. 2023). Trait-based SNP markers help in assessing the genetic diversity of rice genotypes and facilitate the identification of biotic and abiotic stress-tolerant genotypes.

In this context, the present research aimed to screen and identify the rice genotypes with genes/QTLs linked to yield under drought stress and to further use those genotypes in drought-stress breeding programs to develop promising climate-resilient rice varieties for sustainable food production.

Materials and methods

Experimental location and plant materials

The current experiment was conducted at ICAR-NRRI, Cuttack, India, in the year 2023. A set of total one hundred and eighty-two rice genotypes constituted with two *elite* × *elite* breeding populations, *indica* × *japonica* derivatives and 39 tropical *japonica* genotypes were utilized to identify molecularly drought tolerant genotypes.

DNA extraction and genotyping

The 1k-RiCA (Rice Custom Amplicon assay), which was designed using Illumina's TruSeq Custom Amplicon (TSCA) 384 Index Kit technology (https://www.illumina.com) was used for genotyping of the population. The leaf samples collected from all the genotypes were filled separately in each well of DNA extraction plates and oven-dried for twenty hours at a temperature of 40°C. The DNA extraction procedure and genotyping were carried out in accordance with the procedure explained by Intertek India Private Limited (Arbelaez et al. 2019). A total of six gene/ QTLs were used for the drought tolerance screening (Table 1).

Genotype scoring and data analysis

The raw genotypic data was filtered using TASSEL 5.0 (Bradbury et al. 2007). SNP data was filtered by a stringent method, *i.e.,* minor allele frequency (MAF) of >0.5 and a heterozygous proportion of 0.1 were retained. Genotypes were scored using a numerical method by assigning one for favorable alleles and zero for unfavorable ones (Supplementary Tables 1). A cluster diagram was constructed using R software to depict the genetic dissimilarity of evaluated genotypes using scored data for the presence or absence of trait-based SNP markers for drought tolerance. The population structure analysis was carried out using the STRUCTURE v2.3.4 (Pritchard et al. 2000) software by selecting the K values from 1 to 10 with 5 replications. STRUCTURE HARVESTER v6.0 (Earl and von Holdt 2012) was used to estimate the optimum number of sub-populations by plotting Ln P(D) values against the given k value.

Results

Molecular evaluation of all the genotypes for trait-based SNP markers for drought tolerance

Molecular markers are a well-established technique to detect alleles linked to the major phenotypic traits (Xu et al. 2004). The present study was carried out to screen the rice population using six trait-based SNP markers linked to grain yield under drought stress. The 1K RiCA panel of rice which is constituted with highly polymorphic rice markers, has provided insights to screen and identify drought-tolerant genotypes at the molecular level. The results of genotypic screening revealed the presence of vast diversity at the molecular level for drought tolerance among the genotypes used in the study (Fig. 1). Among the total one hundred and eighty-two genotypes, 172 genotypes had a at least one major gene/QTL for drought tolerance. Notably, among the 172 genotypes, thirty-eight genotypes harbored one, one hundred twenty-six genotypes possessed two, five genotypes had three, three genotypes possessed four major gene/QTLs associated with drought tolerance. The result unveiled the presence of the QTLs*viz*., *DTY1.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY4.1* and *qDTY12.1* in 103, 26, 90, 25, 27

Table 1. List of trait-based SNP markers used for molecular screening for drought tolerance in rice genotypes

Gene/ QTL	SNP ID	Marker ID	Favorable allele	Unfavorable allele	References
DTY1.1	snpOS00408	DTY1-1_4	G	T	Ghimire et al. (2012) Vikram et al. (2011)
qDTY2.2	snpOS00413	DTY2-2_2	T	A	Swamy et al. (2013) Palanog et al. (2014)
qDTY3.1	snpOS00424	DTY3-1_2	C	T	Venuprasad et al. (2009)
qDTY3.2	snpOS00419	DTY3-2-IR64_1	A	G	Yadaw et al. (2013)
qDTY4.1	snpOS00427	DTY4-1_2	C	A	Swamy et al. (2013)
qDTY12.1	snpOS00484	DTY12-1_2	A	G	Bernier et al. (2007)

SNP = single nucleotide polymorphisms, QTL = quantitative trait loci

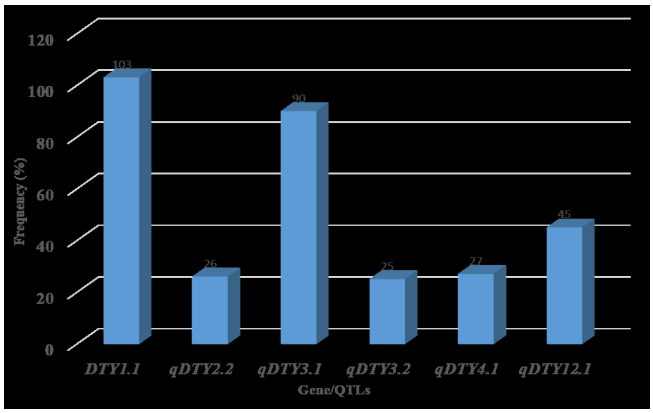

Fig. 1. Frequency distribution in percent of each QTL in all the evaluated genotypes. The results revealed that genes/QTLs *DTY1.1*, *qDTY2.2*, *DTY3.1*, *qDTY3.2*, *qDTY4.1* and *qDTY12.1*found in 103, 26, 90,25, 27 and 45 genotypes corresponding to 57, 14, 49, 14, 15 and 25% of the total evaluated genotypes

and 45 genotypes corresponding to 57, 14, 49, 14, 15 and 25% of the total screened genotypes, respectively.

Cluster analysis based on trait-based SNP markers

The cluster analysis (Fig. 2) differentiated the one hundred and eighty-two genotypes into two major clusters, namely, Cluster 1 (Red color) and Cluster 2 (Blue and green color). The cluster one and cluster two consisted of 90 and 92 genotypes, respectively. Further, cluster 1 was divided into 1A and 1B sub-clusters and the cluster was divided into 2A, 2B and 2C (Blue color) and 2D (Green color). Each sub-cluster explained either the presence/absence of favorable alleles or the unique combination of genes/QTLs within its constituent genotypes.

Structure analysis

The population structure analysis revealed two sub-populations at K=2 among the one hundred and eighty-two rice genotypes studied. The greatest log-likelihood value (K) was detected at K = 2 and indicated that all the one hundred and eighty-two genotypes can be divided into two subpopulations (Fig. 3). This result is in accordance with the result of cluster analysis.

Discussion

Drought is indeed one of the major abiotic stresses that can significantly limit rice grain yield. As per previous research findings, producing one kilogram of rice grains requires 3000-5000 liters of water. Cultivating rice with less water is a global issue and a significant concern for rice-growing countries, including India. One of the major limitations in drought tolerance breeding programs is lack of complete insights into the genetic and molecular mechanisms of drought tolerance in rice. Recent advancements in high throughput phenotyping and molecular markers have led to the screening of numerous genotypes, identifying drought-tolerant genotypes and developing resilient varieties. In order to make this meaningful, we evaluated one hundred and eighty-two rice genotypes at a molecular level to identify sources for drought tolerance genes/QTLs.

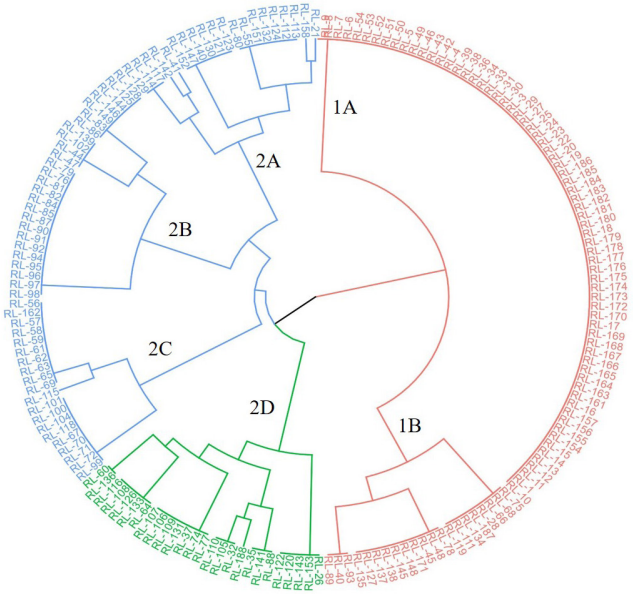

Fig. 2. Clustering of 182 rice genotypes using unweighted pair group method with arithmetic mean (UPGMA). The cluster analysis revealed that all the genotypes were grouped into two major clusters based on the presence or absence of six trait-based gene/QTLs for drought tolerance

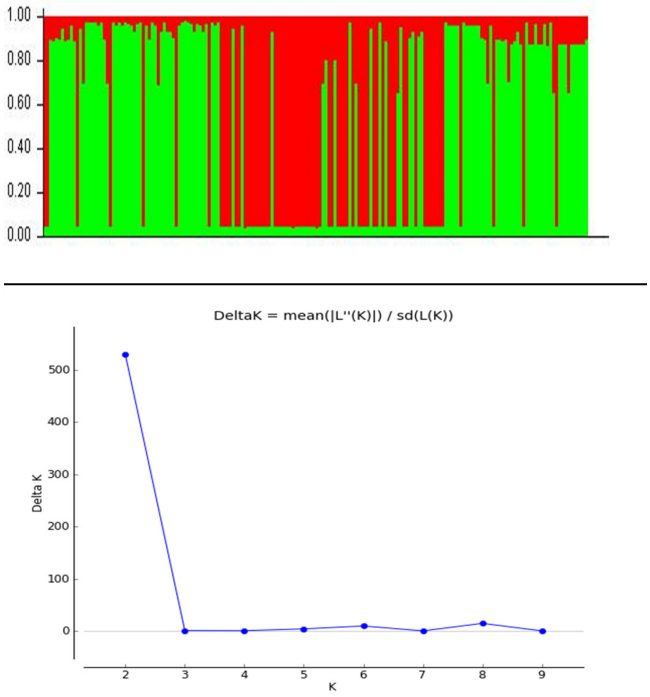

Fig. 3. Population clustering of rice genotypes at estimated membership fraction for K = 2

In the current study, we detected the gene/QTLs *DTY1.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY4.1* and *qDTY12.1* in 57, 14, 49, 14, 15 and 25% of the screened genotypes, respectively. Similarly, Shamsudin et al. (2016) detected *qDTY2.2, qDTY12.1* and *qDTY3.2* in 18, 82 and 36% of the selective pyramided lines, respectively. The genetic diversity analysis of sixty rice accessions by Anupam et al. (2022) has also found *qDTY2.2, qDTY12.1, qDTY4.1* and *qDTY3.2* in 6.67, 43.3, 18.33 and 28.33% of the total evaluated genotypes, respectively. We found *qDTY1.1* in 57% of the genotypes screened. Similarly, Vikram et al. (2011) identified *qDTY1.1* as a major QTL governing grain yield under reproductive drought stress. Therefore, molecular screening of genotypes for the presence of DTY QTLs is a critical step in drought breeding programs for rice. It accelerates the breeding process, improves the efficiency of selecting drought-tolerant genotypes, and ultimately contributes to the development of resilient rice varieties that can thrive in water-scarce environments.

Cluster analysis revealed that genotypes were grouped purely based on either a gene or a combination of genes present in them. Each sub-cluster explained either the presence/absence or the unique combination of genes/QTLs within its constituent genotypes. Sub-cluster 1A was constituted with genotypes possessing *DTY1.1* and *qDTY3.1* and subcluster 1B was constituted with eight genotypes having *DTY1.1*, three genotypes having *qDTY3.1* and ten genotypes not possessing any of the gene/QTLs. The genotypes of subcluster 2A were possessed genes/QTLs *DTY1.1, qDTY3.1, qDTY3.2, qDTY4.1* and *qDTY12.1*, whereas sub-cluster 2B constituted with genotypes possessing *DTY1.1, qDTY3.1* and *qDTY12.1* in different combinations. Similarly, genotypes of subcluster 2C constituted with genotypes having *DTY1.1, qDTY4.1* and *qDTY12.1*, whereas genotypes of sub-cluster 2D possessed *DTY1.1, qDTY2.2, qDTY3.2, qDTY4.1* and *qDTY12.1* in different combinations. Results of population structure analysis also detected only two major subgroups and suggest that the entire population can be segmented into two sub-populations based on either a gene or a combination of genes present in the genotypes. Subpopulations 1 (Green color) and 2 (Red color) comprised 90 and 92 genotypes, respectively. This is in accordance with the result of cluster analysis.

Our study also found a set of genotypes, each with a unique combination of three or four major gene/QTLs. Notably, three genotypes RL-32, RL-105, RL-110 possessed combination of *DTY1.1, qDTY2.2* and *qDTY12.1*, whereas RL-142 possessed *qDTY3.2, qDTY4.1* and *qDTY12.1* and RL-158 possessed *DTY1.1, qDTY3.2* and *qDTY12.1* three gene/QTLs. The genotypes RL-21(*DTY1.1, qDTY3.1, qDTY3.2* and *qDTY12.1*), RL-41(*qDTY3.1, qDTY3.2, qDTY4.1* and *qDTY12.1*) and RL-188 (*DTY1.1, qDTY3.1, qDTY2.2* and *qDTY12.1*) had four major gene/QTLs for drought tolerance, indicating their potential use in the marker-assisted backcross breeding programs, as highlighted in studies by(Shamsudin et al. 2016; Singh et al. 2022), that favorable combinations of QTLs are the best approach towards improving susceptible varieties for drought tolerance. Swamy et al. (2013) also described the reaction of particular combinations of QTLs governing grain yield under drought stress. The pyramiding of QTLs *qDTY3.1*, *qDTY2.2* with *qDTY12.1*remarkably increased the grain yield of the lines processing *qDTY12.1* in the study conducted by Shamsudin et al. (2016). Research findings of (Dixit et al. 2012; 2014) observed that DTY QTLs such as *qDTY3.1* and *qDTY2.2* showed a compatible effect across the seasons under lowland conditions. Therefore, a pyramiding of the rice varieties with a good combination of genes/QTLs with complementary interaction is an effective strategy to increase the performance under drought stress (Sandhu et al. 2017).

The present research indicated the molecular insights of the advanced rice genotypes by presence/absence and either single or combination of drought tolerant genes/QTLs in almost all the genotypes screened. It provides the scope for utilizing these genotypes as a source of drought tolerance in rice varietal improvement for drought conditions or understanding the mechanisms lying under drought stress conditions. The genotypes carrying sum of three (RL-32, RL-105, RL-110, RL-142 and RL-158) or four (RL-21, RL-41 and RL-188) major gene/QTLs for drought tolerance could be further used as donor parents in marker-assisted backcross breeding program for rice drought tolerance.

Supplementary material

Supplementary Table S1 provided with SNP scoring data for all the 182 rice genotypes, www.isgpb.org.

Authors' contributions

Conceptualization (LMR); Designing of the experiments (LMR, RLV, AM, SM); Contribution of experimental materials (RLV, PS); Execution of field/lab experiments and data collection (LMR); Analysis of data and interpretation (LMR, BND); Preparation of the manuscript (LMR, RLV, BND). All authors have read and approved the content of the manuscript.

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Supplementary Table S1. SNP scoring data for all the rice genotypes screened for the presence or absence of trait-based SNP markers

S. No.	Genotype	DTY1.1	DTY2.2	qDTY3.1	DTY3.2	DTY4.1	DTY12.1
1	RL-1	1	0	1	0	0	0
2	RL-10	1	0	1	0	0	0
3	RL-100	1	0	0	0	1	0
4	RL-101	1	0	0	0	1	0
5	RL-102	1	0	0	0	0	1
6	RL-104	1	0	0	0	1	0
7	RL-105	1	1	0	0	0	1
8	RL-106	1	1	0	0	0	0
9	RL-107	1	1	0	0	0	0
10	RL-108	0	1	0	0	0	0
11	RL-109	1	1	0	0	0	0
12	RL-11	1	0	1	0	0	0
13	RL-110	1	1	0	0	0	1
14	RL-111	1	0	0	0	0	0
15	RL-112	0	0	0	1	0	1
16	RL-113	0	0	0	1	0	1
17	RL-114	0	0	0	1	1	0
18	RL-115	0	0	0	0	1	1
19	RL-116	0	1	0	0	0	0
20	RL-117	0	0	0	1	1	0
21	RL-118	1	0	0	0	1	0
22	RL-119	1	0	0	0	0	0
23	RL-12	1	0	1	0	0	0
24	RL-120	0	1	0	1	0	0
25	RL-121	0	0	0	1	0	0
26	RL-122	0	1	0	1	0	0
27	RL-123	0	0	0	1	0	0
28	RL-124	0	0	0	1	0	1
29	RL-125	0	0	0	0	0	1
30	RL-126	0	1	0	0	0	0
31	RL-127	0	0	0	0	0	0
32	RL-128	0	0	0	0	0	1
33	RL-129	0	0	0	1	1	0
34	RL-13	1	0	1	0	0	0
35	RL-130	0	0	0	1	0	0
36	RL-131	1	1	0	0	0	0
37	RL-132	0	0	0	1	0	1
38	RL-133	0	1	0	0	0	0
39	RL-134	1	0	0	0	0	0
40	RL-135	0	0	0	0	0	0
41	RL-136	0	1	0	0	1	0
42	RL-137	0	0	0	0	0	0
43	RL-138	0	0	0	0	0	0
44	RL-139	1	0	0	0	0	1

45	RL-14	1	0	1	0	0	0
46	RL-140	0	0	0	1	0	0
47	RL-141	0	1	0	0	0	1
48	RL-142	0	0	0	1	1	1
49	RL-143	0	1	0	1	0	0
50	RL-144	0	0	0	0	0	1
51	RL-145	0	0	0	0	0	0
52	RL-146	0	0	0	0	0	1
53	RL-147	0	0	0	1	0	0
54	RL-148	0	0	0	0	0	0
55	RL-149	0	0	0	0	0	1
56	RL-15	1	0	1	0	0	0
57	RL-151	0	0	0	1	0	1
58	RL-152	0	0	0	1	0	0
59	RL-153	0	1	0	1	0	0
60	RL-154	1	0	1	0	0	0
61	RL-155	1	0	1	0	0	0
62	RL-156	1	0	1	0	0	0
63	RL-157	1	0	1	0	0	0
64	RL-158	1	0	0	1	0	1
65	RL-16	1	0	1	0	0	0
66	RL-161	1	0	1	0	0	0
67	RL-162	0	0	0	0	1	0
68	RL-163	1	0	1	0	0	0
69	RL-164	1	0	1	0	0	0
70	RL-165	1	0	1	0	0	0
71	RL-166	1	0	1	0	0	0
72	RL-167	1	0	1	0	0	0
73	RL-168	1	0	1	0	0	0
74	RL-169	1	0	1	0	0	0
75	RL-17	1	0	1	0	0	0
76	RL-170	1	0	1	0	0	0
77	RL-171	0	0	0	0	0	0
78	RL-172	1	0	1	0	0	0
79	RL-173	1	0	1	0	0	0
80	RL-174	1	0	1	0	0	0
81	RL-175	1	0	1	0	0	0
82	RL-176	1	0	1	0	0	0
83	RL-177	1	0	1	0	0	0
84	RL-178	1	0	1	0	0	0
85	RL-179	1	0	1	0	0	0
86	RL-18	1	0	1	0	0	0
87	RL-180	1	0	1	0	0	0
88	RL-181	1	0	1	0	0	0
89	RL-182	1	0	1	0	0	0
90	RL-183	1	0	1	0	0	0

91	RL-184	1	0	1	0	0	0
92	RL-185	1	0	1	0	0	0
93	RL-186	1	0	1	0	0	0
94	RL-187	1	0	0	0	0	0
95	RL-188	1	1	1	0	0	1
96	RL-19	1	0	1	0	0	0
97	RL-20	1	0	1	0	0	0
98	RL-21	1	0	1	1	0	1
99	RL-22	1	0	1	0	0	0
100	RL-23	1	0	1	0	0	0
101	RL-24	1	0	1	0	0	0
102	RL-25	1	0	1	0	0	0
103	RL-26	0	1	0	1	0	0
104	RL-27	1	0	1	0	0	0
105	RL-28	1	0	0	0	0	0
106	RL-29	1	0	1	0	0	0
107	RL-3	1	0	1	0	0	0
108	RL-30	1	0	1	0	0	0
109	RL-31	1	0	1	0	0	0
110	RL-32	1	1	0	0	0	1
111	RL-33	1	0	1	0	0	0
112	RL-34	1	0	1	0	0	0
113	RL-35	0	1	0	0	0	1
114	RL-36	1	0	1	0	0	0
115	RL-37	1	1	0	0	0	0
116	RL-38	1	0	1	0	0	0
117	RL-39	1	0	1	0	0	0
118	RL-4	1	0	1	0	0	0
119	RL-40	0	0	1	0	0	0
120	RL-41	0	0	1	1	1	1
121	RL-42	1	0	1	0	0	0
122	RL-43	1	0	1	0	0	0
123	RL-44	1	0	0	0	0	1
124	RL-45	0	0	0	0	0	0
125	RL-46	1	0	1	0	0	0
126	RL-47	1	0	0	0	0	1
127	RL-48	0	0	0	0	0	0
128	RL-49	1	0	1	0	0	0
129	RL-5	1	0	1	0	0	0
130	RL-50	1	0	1	0	0	0
131	RL-51	1	0	1	0	0	0
132	RL-52	1	0	1	0	0	0
133	RL-53	1	0	1	0	0	0
134	RL-54	1	0	1	0	0	0
135	RL-55	0	0	0	1	0	1
136	RL-56	0	0	0	0	1	0

137	RL-57	0	0	0	0	1	0
138	RL-58	0	0	0	0	1	0
139	RL-59	0	0	0	0	1	0
140	RL-6	1	0	1	0	0	0
141	RL-60	0	1	0	0	1	0
142	RL-61	0	0	0	0	1	0
143	RL-62	0	0	0	0	1	0
144	RL-63	0	0	0	0	1	0
145	RL-64	0	1	0	0	0	0
146	RL-65	0	0	0	0	1	0
147	RL-66	1	0	0	0	0	1
148	RL-67	1	0	0	0	1	0
149	RL-68	1	0	0	0	0	0
150	RL-69	0	0	0	0	1	0
151	RL-7	1	0	1	0	0	0
152	RL-70	1	0	0	0	1	0
153	RL-71	1	0	0	0	1	0
154	RL-72	1	0	0	0	1	0
155	RL-74	1	1	0	0	0	0
156	RL-75	1	0	0	0	0	0
157	RL-76	0	0	1	0	0	1
158	RL-77	1	1	0	0	0	0
159	RL-78	0	0	0	0	0	0
160	RL-79	0	0	1	0	0	1
161	RL-8	1	0	1	0	0	0
162	RL-80	0	0	0	1	0	1
163	RL-81	0	0	1	0	0	1
164	RL-82	0	0	1	0	0	1
165	RL-83	0	0	0	0	0	1
166	RL-84	0	0	1	0	0	1
167	RL-85	0	0	1	0	0	1
168	RL-86	0	0	0	0	0	1
169	RL-87	0	0	1	0	0	1
170	RL-88	0	1	0	0	0	1
171	RL-89	0	0	1	0	0	0
172	RL-9	1	0	1	0	0	0
173	RL-90	0	0	1	0	0	1
174	RL-91	0	0	1	0	0	1
175	RL-92	0	0	1	0	0	1
176	RL-93	0	0	1	0	0	0
177	RL-94	0	0	1	0	0	1
178	RL-95	0	0	1	0	0	1
179	RL-96	0	0	1	0	0	1
180	RL-97	0	0	1	0	0	1
181	RL-98	0	0	1	0	0	1
182	RL-99	1	0	0	0	1	0