

Genetic diversity in allelopathic rice accessions (*Oryza sativa* L.)

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to estimating genetic diversity among 57 allelopathic rice accessions (*Oryza sativa* L.). There were 59 polymorphic bands in the 85 RAPD loci within the tested accessions, and the percentage of polymorphic bands (PPB) was 69.4%. The result from the clustering analysis by Unweighted Pair Group Method Arithmetic Average (UMGMA) indicated that those accessions from the same geographical location were clustered into one group. It was also found that some rice accessions with higher allelopathic potential were clustered together, such as IAC25, IAC47 and IAC120 implying that the genes conferring allelopathy in those rice accessions might be isologous. However, some rice accessions with different allelopathic potential clustered into the same group performed lower level of generic polymorphism which was attributed to oriented selection for other traits in breeding program. This offers us a genetic pool for the selection of rice cultivars with high allelopathic ability.

Key Words

Allelopathic rice accessions; *Oryza sativa* L., Genetic diversity; RAPD

Introduction

Weeds are one of the most important causes of yields losses in rice. In the upland rice ecosystem, yield loss estimates from weed infestation have ranged from 30 to 100% (Luoise 2001). With the increasing of labor-cost increasing and the availability and efficacy of herbicide, herbicide use has increased exponentially in Asian rice production (Naylor 1996). Despite the overwhelming economic benefit, the heavy reliance on chemical herbicides is considered a potential threat to public health and to the environment (Jiwan and Gates, 1994). Furthermore, the appearance of herbicides-resistance weeds is an increasing problem (Boutsalis and Powles, 1995). These factors have prompted research into alternative means of weed control. (Motiul Quader et al, 2001).

Allelopathy is defined as "direct or indirect (harmful or beneficial) effects of a plant, on another plant through the release of compounds that escapes into the environment" (Rice, 1984). Three seasons of field experience finally led to the conclusion that allelopathy could explain 34% of the variation among cultivars in the ability to suppress weeds (Olofsdotter et al. 1999).

Allelopathic potential has been identified in most major cereal crops (Lovett and Hout, 1995). Based on the selection for rice accessions, the assessment of genetic diversity became necessary when a significant level of intraaccession variability in allelopathic expression became evident (Motiul Quader et al, 2001).

Methods and Materials

Plant Materials

Fifty-seven rice accessions of *Oryza sativa* L. introduced from American, Korea, Brazil, China and the International Rice Research Institute (IRRI), were screened for allelopathic potential. Barnyardgrass (*Echinochloa crus-galli* L.) was used as a receiver plant.

Screening for allelopathic potential

The procedure was adapted from the relay seedling technique (Navarez and Olofedotter 1996). 0.5% water agar was used as growth medium after compared with Perlite (the data unpublished). The root length of barnyardgrass was recorded.

DNA extraction and RAPD analysis

All seeds were grown in Petri dishes containing 0.5% water agar under sterile conditions. Genomic DNA were extracted from duplicate samples from a single seedling by the method of Weining et al.(1994). The polymerase chain reaction (PCR) was performed in a 15µl reaction mixture containing 10ng template DNA, 1.5µl 10²buffer, 200µmol/L dNTPs, 1.5µl Taq DNA polymerase and 1ng 10-mer primer. Amplification program is : 30s at 94^o ; 30s at 40^o ; 90s at 72^o ; 40 cycles, 10min at 72^o then stored at 4^o.

Statistical Analysis

The root length of barnyardgrass was transferred into Inhibition Ratio (IR), i.e. $IR = (1 - TR/CK) \times 100\%$, while TR represented the treatment, CK was the control.

Genetic data analyses were performed by using the computer package TFPMPG 1.3(Miller et al.1997).The dendrograms was constructed by the unweighted pair group method (UPGMA)(Sneath and Sokal,1973).

Results

Screening for allelopathic potential

Of 57 accessions, 5 rice cultivars, such as Iguape Cateto, PI312777, Azucena, Taichung Native 1 and IAC25, demonstrated over 50% inhibition of barnyardgrass root growth. IRs of 12 cultivars ranged from 40% to 50%, that of 21 cultivars from 30% to 40%, 13 cultivars from 20% to 30%, while IRs of 6 cultivars were less than 20%(Table 1). The distribution of allelopathic activity in rice accessions tested was normal(Fig 1).

Table 1: IRs of rice allelopathy on the root of barnyardgrass*

No	Rice accession	Origin	IR (%)	No	Rice accession	Origin	IR (%)
1	Iguape Cateto	Brazil	58.4 \pm 1.6	30	Chaoerzhan	China	33.3 \pm 6.3
2	PI312777	America	56.8 \pm 2.3	31	Sanyizhaozhan	China	32.7 \pm 6.7
3	Azucena	The Philippines	53.9 \pm 1.4	32	Wab56-125	Ivory Coast	32.5 \pm 4.1
4	Taichung Native 1	Taiwan	50.2 \pm 8.9	33	Polha Murcha	Brazil	32.0 \pm 6.6
5	IAC25	Brazil	50.0 \pm 4.7	34	Qisanzhan	China	31.8 \pm 7.5
6	AU257	Bangladesh	48.4 \pm 0.6	35	Dourado Pecoco	Brazil	31.8 \pm 3.5

7	Red Rice5	China	48.2?6.8	36	Bala	India	31.6?6.7
8	Batatais	Brazil	47.5?5.2	37	Arroz de campos	Cuba	30.7?7.9
9	IAC120	Brazil	46.7?7.4	38	Shuangzhan 2	China	30.4?7.5
10	Co39	India	45.2?2.6	39	Fengaizhan	China	29.1?5.8
11	IAC47	Brazil	45.1?7.5	40	IR721413	The Philippines	28.9?4.7
12	IR72417-3R-8-2	The Philippines	44.5?13.2	41	Qidaizhan	China	28.9?8.8
13	Yehuazhan	China	43.6?5.9	42	IR73384	The Philippines	27.7?9.4
14	IR70617	The Philippines	43.2?6.7	43	IR64	The Philippines	27.7?5.3
15	Jingyouzhan	China	41.1?8.6	44	Xinsimiao	China	27.3?1.3
16	IAC164	Brazil	41.1?6.9	45	Daishuzhan	China	27.0?4.3
17	Mafeng 1	China	40.9?10.1	46	Qingxiangzhan	China	26.8?6.1
18	189Taizhong 189	China	39.3?8.6	47	IR62266-42-6-2	The Philippines	25.9?4.0
19	Dinorado	The Philippines	39.3?2.2	48	IR65907-116-1-B	The Philippines	24.1?9.1
20	Vandana	Colombia	38.2?5.6	49	Moroberekan	Guinea	23.2?6.6
21	IAC165	Brazil	37.5?1.1	50	IR60080-46A	The Philippines	21.4?4.1
22	IR56	The Philippines	36.6?4.7	51	IR72412	The Philippines	20.2?7.8
23	Shuangmeizhan	China	36.4?6.4	52	Zhengyou 1	China	19.5?1.9
24	IR70651	The Philippines	35.7?2.4	53	IR55423-01	The Philippines	18.4?2.5
25	IR36	The Philippines	34.5?4.3	54	Pratao Precoce	Brazil	18.2?2.6

26	Dee Geo Woo Gen	Taiwan	34.3?3.8	55	Aisanruzhan	China	17.7?4.6
27	Muxiang 25	China	34.1?3.1	56	Dular	America	14.1?2.1
28	IR73382	The Philippines	33.9?2.8	57	Lemont	America	10.9?3.2
29	IR71331	The Philippines	33.3?9.4	CK			0.0

*CK, Control= *Echinochloa crusgalli* L. grown alone

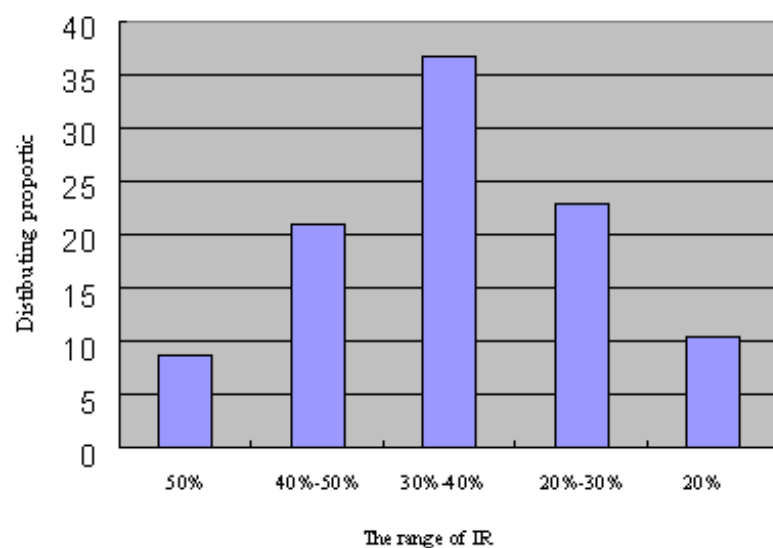


Fig.1(Left) The distribution proportion of rice accessions in different interval of IR value

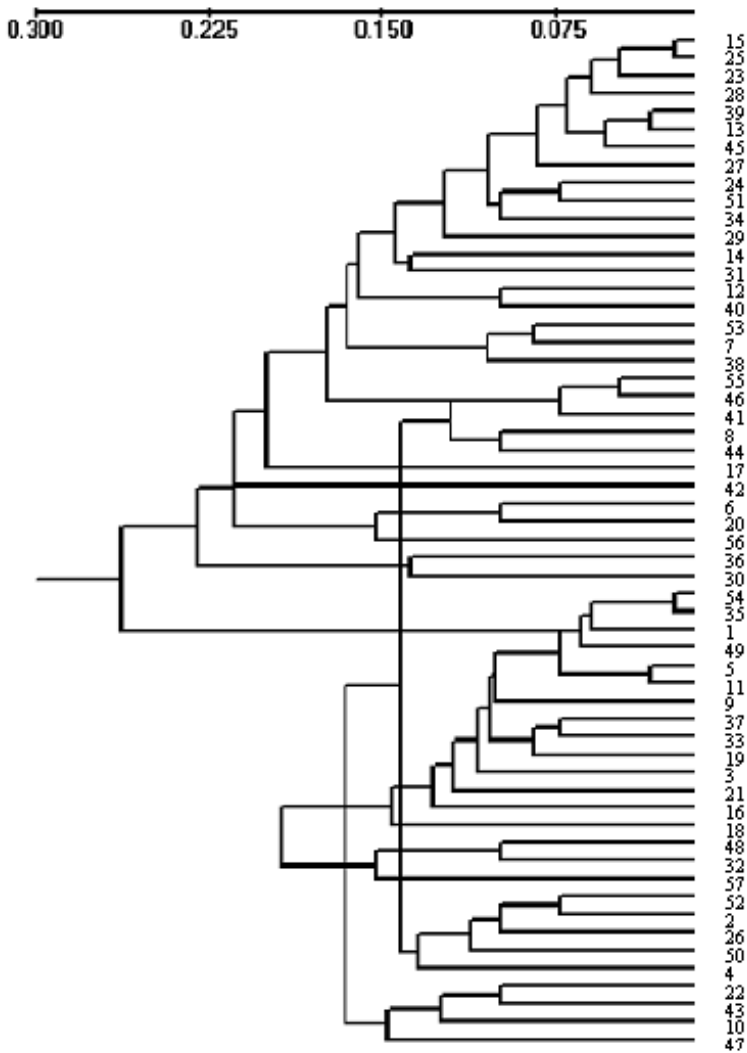


Fig.2 (Right) The cluster analysis on allelopathic rice as determined by UPGMA from RAPD

Genetic diversity in 57 accessions

12 primers were selected from a previous study on the basis of polymorphisms revealed in 5 rice accessions. The primers were used to detect the polymorphism of 57 rice accessions (Figure 3). The total of 85 bands were scorable, of which 59 were polymorphic, and the percentage of polymorphic bands(PPB) was 69.4%.

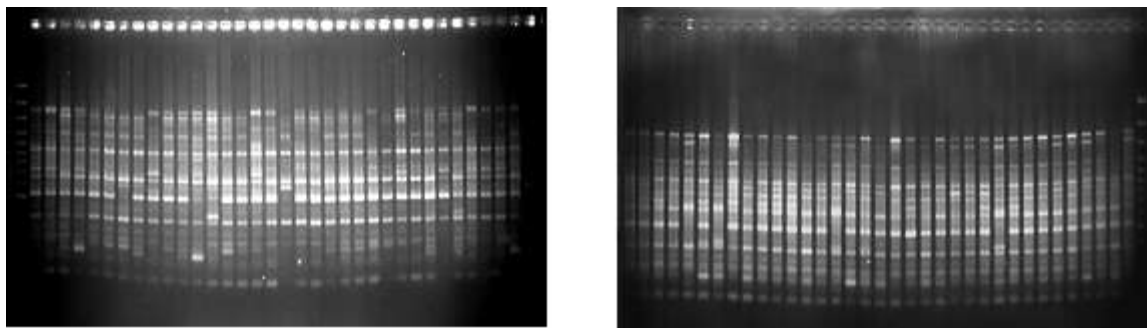


Fig.3 DNA fragment amplified by primer 1409

Clustering

From $GD=0.1875$, 57 rice accessions were grouped into eight main group(Fig.2). The first group was composed of 31 cultivars, of which 14 were from Mainland China, 13 from the Philippines, 2 from Taiwan, 1 from American and 1 from India. In this group, 8 cultivars showed over 40% of inhibitory effect on the root of baryardgrass. The sixth group included 6 accessions introduced from Brazil. Three cultivars, IAC25(5), IAC47(11) and IAC120(9), which all showed high allelopathic potential on barnyardgrass, were clustered together. Another three cultivars, Iguape Cateto(1), Dourado Pecoce(35) and Pratao Pecoce(54) were grouped into one subgroup, performing that the inhibition ratio (IR) were 58.4%, 31.8% and 18.2% respectively.

Discussion

In this study, the genetic polymorphism of allelopathic rice detected by RAPD approach indicated that those accessions from the same geographical location were clustered into one group. It was also found that some rice accessions with higher allelopathic potential were clustered together, implying that the genes conferring allelopathy in those rice accessions might be isolocus. However, some rice accessions with different allelopathic potential clustered into the same group, such as Iguape Cateto(1), Dourado Pecoce(35) and Pratao Pecoce(54), performed lower level of generic polymorphism which was attributed to oriented selection for other traits in breeding program. Bustos et al (1998) have successfully used RAPD markers to investigate the genetic diversity within and among wild populations of species of the genus *Hordeum* (Poaceae). With careful screening and replication, M.Quader et al (2001) observed high level of intraaccession genetic diversity (4-24%) in accessions of *Triticum Speltoides* by using RAPD.

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