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Variation of Grain Traits in Landraces of Rice Collected from Vietnam

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SUMMARY

A total of 437 landraces of rice from Vietnam were analyzed for total seed protein by SDS-PAGE and phenol reaction. The different types of glutelin α subunits were detected. The level of wx protein with 60kDa molecular weight was divided into 3 groups, corresponding to non-glutinous, intermediate and glutinous starch types. Based on the variation in seed storage protein and wx protein, landraces were classified into 7 groups. Frequency distribution of types A and B of glutelin α subunits changed with the latitude at which rice landraces were collected. Geographical cline for phenol reaction was detected.

Key Words : Rice, Vietnamese landraces, Seed protein, wx protein, SDS-PAGE

Introduction

Vietnam shares common borders with China, Laos and Cambodia and is included in the Indo-Malayan center of diversity for many crops. Northern Vietnam which is adjacent to Yunnan province in China is the east edge of a center of diversity for cultivated rice and there are many ethnic groups.

Missions were undertaken to collect landreaces of rice in northern, central and southern Vietnam in 1994. Esterases isozyme analysis revealed that 11 esterase genotypes out of 12 possible genotypes could be detected in the landraces from northwestern Vietnam which still has diverse rice germplasm (Okuno et al. unpublished data). This report deals with variation in seed protein, *wx* protein and phenol reaction of Vietnamese rice landraces.

Materials and Methods

A total of 437 landraces collected by IPGRI/ NIAR/ DAST collaborative exploration missions were used in this study. These samples were collected from 18 provinces in 5 regions of Vietnam.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of crude protein, including wx protein

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extracted from seed samples was carried out according to the method of Laemmli(2). Two grams of each ground sample were added to 4 mℓ of sample buffer(0.05M tris-HCL with pH8.0) including 1.5% sodium dodecyl sulfate(SDS), 0.1% 2-mercatpoethanol and 5M urea.

Seed storage proteins from each sample were extracted to 20 min and were centrifuged for 15 min to remove debris. The seed storage proteins were separated by SDS slab gel eletrophoresis for 8 hr. at a constant current 150V per gel using polyacrylamide gel(14%) including 5M urea with a stacking gel(7%). After loading of eletrophoresis, gel was stained in a soultion (0.2% coomassie brilliant blue R250), was destained in a destained solution(70% ethanol and 20% acetic acid).

Several seeds of each landrace were soaked in 1.5% phenol solution at 30 C for 2 days and were then evaluated for positive(intensity +++, ++ and +) and negative(-) reactions.

Results and Discussion

Electrophoretic variation in seed storage protein and wx protein

Variation in electrophoregrams of seed protein was observed in glutelin α subunits and *wx* protein with 60kDa molecular weight (Fig. 1). Based on the variation in glutelin α subunits(α-1, α-2, α-3), landraces were classified into 3 different types. Type A has all the subunits and α-2 subunits were found in types A and B. Type C has no α-1 subunit and the same pattern of α-2 and α-3 subuntis as type B. Kagawa et al(1) reported 2 types of glutelin subunits in rice and one of these showed deletion of α-3 subunit in indica varieties. The α-1 subunit deficient type C was found for the first time in this study.

All landraces could be classified into 3 types based on the level of wx protein: nonglutinous glutinous and intermediate types. More than 60% of samples collected from northwestern Vietnam were found glutinous, whereas about 70% of samples from central and southern Vietnam were non-glutinous or intermediate types. Considering the results of both glutelin α subunits patterns and wx protein, landraces can be classified into 7 groups; type A1(α-1, α-2, α-3-higher; non-glutinous), type A2(α-1, α-2, α-3-higher; intermediate), type A3(α-1, α-2, α-3-higher; glutinous) type B1(α-1, α-2, α-3-lower; non-glutinous) type B2(α-1, α-2, α-3-lower; intermediate), type B3(α-1, α-2, α-3-lower; glutionus) and type C1(α-1, α-2, α-3-lower; non-glutinous).

Geographical distribution of the above 7 types was observed (Table 1, 2 and Fig.2).

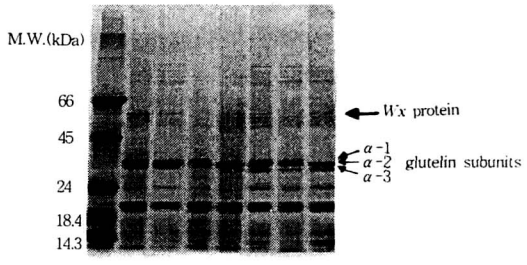

Fig. 1. Variation in electrophoregrams of seed protein from Vietnamese rice landraces.

A1: α-1, α-2, α-3 (higher); non-glutinous
A2: α-1, α-2, α-3 (higher); intermediate
A3: α-1, α-2, α-3 (higher); glutinous
B1: α-1, α-2, α-3 (lower); non-glutinous
B2: α-1, α-2, α-3 (lower); intermediate
B3: α-1, α-2, α-3 (lower); glutinous
C1: α-2, α-3 (lower); non-glutinous

The difference between the two different types is the molecular weight of glutelin α subunit. This latitudal difference in molecular weight of α subunit may be due to mutation of the structural gene which encodes glutelin α subunit during the domestication of rice landraces.

Table 1. Regional variation in electrophoregrams of total seed protein of Vietnamese rice landraces

Regions	Electrophoregrams							
	A1	A3	A2	B1	B3	B2	C1	Total
Northwest	22	12	32	17	37	63	0	183
Central	15	3	11	10	0	5	1	45
Highland	23	4	24	8	1	5	0	65
South	37	1	8	17	1	2	0	66
Southeast	30	7	9	15	2	0	0	63
Total	127	27	84	67	41	75	1	422

Landraces having type A are widespread throughout central and southern Vietnam, while landraces with type B predominated in the northwest.

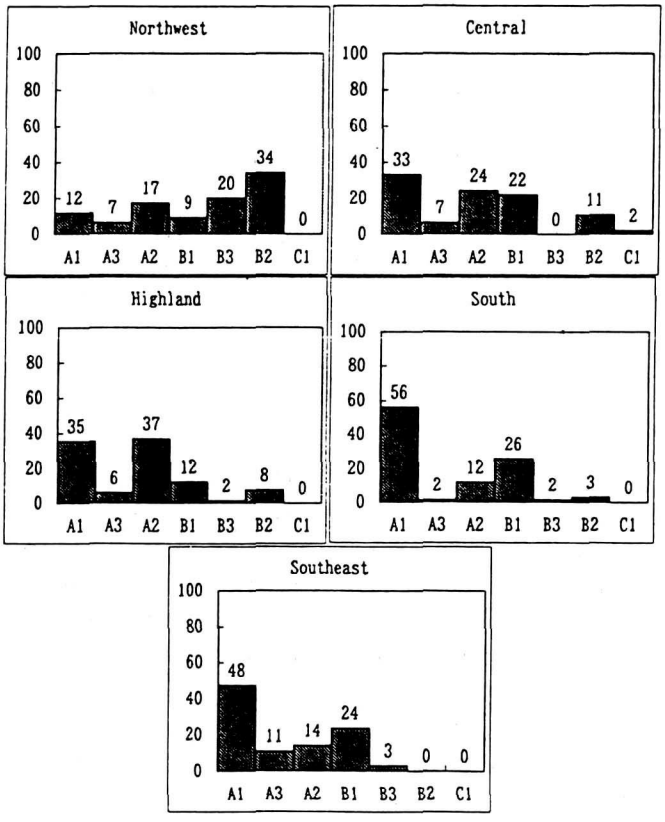

Fig. 2. Geographical variation in glutelin *α* subunits and *wx* protein of Vietnamese rice landraces.

Frequency distribution of types A and B changed with the latitude at which rice landraces were collected.

Table 2. Geographical variation in electrophoregram of total seed protein of landraces.

Province	Electrophoregrams patterns							
	A1	A2	A3	B1	B2	B3	C1	Total
Thua Thien Hue	1	1	0	2	0	0	0	4
Da Nang	5	2	0	2	0	0	1	10
Quang Ngai	4	0	0	1	1	0	0	6
Binh Dinh	3	2	2	0	0	0	0	7
Tuy Hoa	2	6	1	0	2	0	0	11
Lam Dong	0	0	0	5	2	0	0	7
An Giang	15	0	0	12	1	1	0	29
Ha Tien	1	3	0	3	1	0	0	8
Rach Gia	14	3	1	2	0	0	0	20
Dong Thap	7	2	0	0	0	0	0	9
Tay Ninh	6	5	0	12	0	0	0	23
Song Be	24	4	7	3	0	2	0	40
Dac Lac	20	19	2	8	5	1	0	65
Gia Lai	2	3	1	0	0	0	0	6
Kon Tum	1	2	1	0	0	0	0	4
Son La	6	7	2	0	14	3	0	32
Lai Chau	11	22	10	16	46	24	0	129
Lao Cai	5	3	0	1	3	10	0	22
Total	127	27	84	67	41	75	1	422

wx protein is a gene product of wx locus on chromosome 6 of rice and is responsible of amylose production in endosperm and pollen grains. Intermediate type having lower content of amylose have been found in rice, foxtail millet and grain amaranth. A regulatory mechanism of intermediate type may be due to a trans-acting like element, double gene found by Okuno et al(3,4) in 1983 and 1985, or wx alleles at wx locus(5). The genetic mechanism for intermediate level of wx protein and low amylose content should be studied.

Variation in phenol reaction

Landraces were divided two major groups; positive and negative, and also subdivided 3 groups based on the reaction intensity. About 60% of landraces from the northwest were showed negative reaction to phenol, whereas about 50% of landraces from central and about 90% of landraces from the south reacted positively. The difference in phenol reaction was very clear between northern and southern landraces. Geographical cline for phenol reaction was found (Fig. 3). In addition to negative phenol reaction, landraces with esterase genotype found in Japonica rice predominated among the samples collected in northwestern Vietnam (Okuno et al. unpublished data).

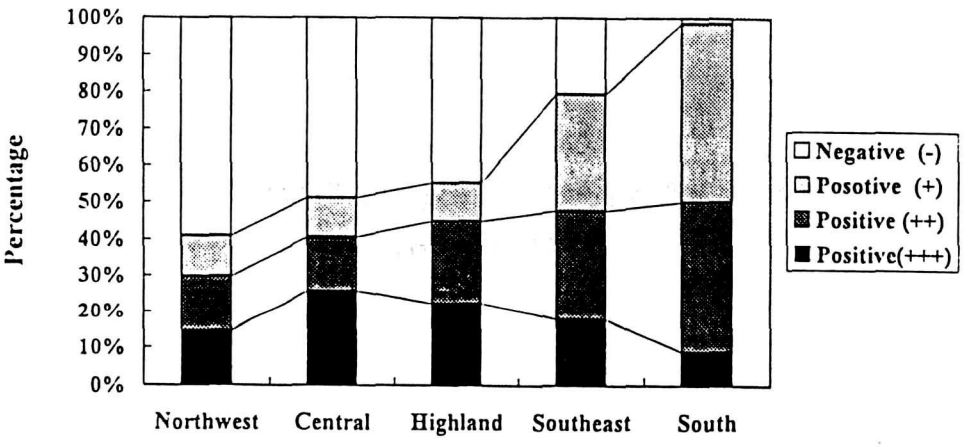

Fig. 3. Georaphical variation in phenol reaction of Vietnamese rice landraces.

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베트남 지방수집종 벼의 종실특성변이

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요 약 : 베트남으로부터 수집된 총 437 벼 지역종을 사용하여 총 종자 단백질을 분석하기 위하여 SDS- PAGE와 phenol 반응을 수행하였다. 세 가지 다른 형태의 glutelin α subunit이 검출되었다. 60kDa의 분자량인 wx 단백질 수준은 세 그룹으로 분리되었는데, 이는 non-glutelin, intermediate, 그리고 glutelin 전분 유형이다. 종자 저장 단백질과 wx 단백질의 변이에 의한 실험결과 지역종은 7그룹으로 구분된다. Glutelin α subunit의 A와 B 형의 빈도분포는 벼 지역종이 수집된 위도에 따라서 변하였다. 페놀 반응에 대한 지리학적인 구배가 검출되었다.