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The diversity of some local upland rice cultivars in Northern of Vietnam

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Abstract— **Droughts have been occurring commonly in the recent years while upland rice became potential crops because of its high drought tolerance. In this study, we have evaluated the diversity of some local upland rice cultivars in Northern Vietnam in order to preserving and developing the genetic resources of upland rice. Forty seven local upland cultivars were collected from mountainous provinces in Northern Vietnam and classified into two subspecies: *indica* (33 cultivars) and *japonica* (14 cultivars). We have evaluated the diversity of these cultivars by studying some morphological and qualitative characteristics of grains. Out of these 47 cultivars, 12 cultivars were selected to evaluate the genetic diversity by RAPD technique. The genetic similarity and different coefficients of these cultivars were determined by using NTSYSpc-2.02i program. The dendrogram is established with four groups and the genetic distances between cultivars range from 7.69% to 34.0%. The genetic diversity coefficient of these cultivars is 52.37%. The reactivity for drought of these 12 upland rice cultivars was shown in the diversity of drought tolerant phenotype. These cultivars were divided into 4 groups according to the relative drought tolerance index. The difference coefficients range from 1.003% to 9.394% and the phenotypic diversity coefficient is 73.15%.**

Keywords-upland rice; diversity; Northern Vietnam; RAPD; drought tolerance.

I. INTRODUCTION

Rice occupies the most important position in the global food system. About half of the world's population, predominantly in Asia, depends on rice as a primary dietary staple [8]. In Asia, most rice is grown under wetland conditions [1]. However, droughts have occurred commonly in the recent years as a result of global warming and climate change. This causes a serious shortage of water supply for growing rice and leads to severe damages in the quality and yield of rice crops. In this situation, upland rice, with its high drought tolerance, has become potential crops. Upland rice also has many precious traits such as good quality rice, high pestilent insect resistance possibility and short growing season.

In Vietnam, upland rice is mainly cultivated in Northern mountainous regions and Central Highlands. Due to the development and modernization of agricultural production in mountainous regions, many upland rice cultivars are degenerated and in danger of losing. In order to preserve and exploit full potential of upland rice in Vietnam, it is necessary to study systematically, providing the basis for selective breeding high quality and drought tolerance upland rice cultivars. In this study, we present results on collection, classification and analysis the diversity of 47 local upland rice cultivars. Besides, we also evaluated the phenotypic diversity for drought tolerance and genetic diversity of 12 cultivars and the obtained result of this study shall be used as basis information for breeding drought tolerant rice cultivars, having a part in preserving and developing the genetic resources of upland rice

II. MATERIALS AND METHOD

Forty seven upland rice cultivars were collected in mountainous provinces in Northern Vietnam. Local name of these cultivars are shown in table I.

Classification of these upland rice cultivars into two subspecies - *japonica* and *indica* according to Chang (1976) [2].

The morphological and qualitative characteristics were analyzed based on scales in Standard evaluation system for rice (IRRI, 1996) [4].

Protein content was determined according to Lowry [6]. Lipid content was determined by using Gerhardt Soxhlet extraction system (Gerhardt, Germany).

Total DNA was isolated by using method of Foolad et al. (1995) [3] with CTAB extraction buffer 4.0%. RAPD reactions for genome analysis using random primers [9,10] were carried out in 25 ml solution included 1X PCR buffer; 2.5 mM $MgCl_2$; 100 µl dNTPs; 200 mM primers; 0.125 unit of *Taq* polymerase and 5 ng DNA template. The reactions were carried out in GeneAmp® PCR System 9700 (Applied Biosystem, USA). The RAPD products were analyzed via electrophoresis in 1.8% agarose gel. Analysis of phenotypic diversity for drought tolerance was based on survey of the reactivity of these upland rice cultivars for the impact of drought, through the expression of 20-day-old plants under artificial drought condition. The relative drought tolerance index was determined according to Le Tran Binh et al (1998) [5].

The RAPD data and reactivity of these upland rice cultivars for drought were analyzed using NTSYSpc-2.02i program (Applied Biostatistics Inc., USA., 1998). The diversity coefficient was given by:

$$H = 1 - \sum_{i=1}^{k} P_i^2$$

where H is the diversity coefficient, P_i is the repeat allele frequency, i = 1 – k [7].

III. RESULTS AND DISCUSION

A. Collection and classification of some local upland rice cultivars

Forty seven upland rice cultivars were collected in 5 mountainous provinces (Son La, Bac Kan, Cao Bang, Thai Nguyen, Ha Giang) in Northern Vietnam. There are 33 cultivars belong to subspecies *indica* and 14 cultivars belong to subspecies *japonica* as shown in Table II.

B. Evaluation of some morphological and qualitative characteristics of grains

We have analyzed some morphological and qualitative characteristics of grains, such as length – width ratio, awning, 1000-grain weight, lemma and palea color, bran color, chalkiness of endosperm and scent (Table II). Scoring of these characteristics (except for the 1000-grain weight) are based on scales in Standard evaluation system for rice (IRRI, 1996) [10]. The scales are showed in Table I. The diversity of these upland rice cultivars reflected in the diversity of morphological and qualitative characteristics of grains. These results is the basis for evaluation of the quality of upland rice cultivars and preservation and selection the high quality cultivars as well.

C. Protein and lipid content of grains

The analytical results are shown in Table II and it indicated that the is difference in protein and lipid content between these cultivars. These results were also considered as an important in evaluation of the quality of upland rice cultivars.

TABLE I. THE STANDARD EVALUATION SCALES FOR RICE ACCORDING TO IRRI

Characteristic	**Code**	
	Trait	***Scale***
Length – width ratio	1	Over 3.0
	3	2.1 to 3.0
	5	1.1 to 2.0
	9	Less than 1.1
Lemma and palea color	0	Straw
	1	Gold and gold furrows on straw background
	2	Brown spots on straw
	3	Brown furrows on straw
	4	Brown (tawny)
	5	Reddish to light purple
	9	Black
	10	White
Bran color	1	White
	2	Light brown
	3	Speckled brown
	4	Brown
	5	Red
	7	Purple
Awning	0	Absent
	1	Short and partly awned
	5	Short and fully awned
	9	Long and fully awned
Chalkiness of endosperm	0	None
	1	Small (less than 10%)
	2	Medium (11% to 20%)
	3	Large (more than 20%)
Scent	0	Unscented
	1	Lightly scented
	2	Scented

TABLE II. THE CHARACTERISTICS OF FORTY SEVEN LOCAL UPLAND RICE CULTIVARS

No	Local name	Classification	Morphological and qualitative characteristics of grain							Protein and lipid content (% dry matter)	
			Length – width ratio	*1000 grain weight* (g)	*Lemma and Palea color*	*Awn*	*Bran color*	*Chalkiness of Endosperm*	*Scent*	*Protein*	*Lipid*
1	Mo khao	*indica*	1	25.70 ± 0.28	0	0	2	3	0	4.67 ± 0.09	1.23 ± 0.211
2	Khau chat	*indica*	1	28.54 ± 0.87	10	1	2	0	1	5.50 ± 0.27	1.67 ± 0.204
3	Beo giang	*indica*	1	30.32 ± 1.14	4	9	2	0	0	5.96 ± 0.41	1.41 ± 0.204
4	Khau bau	*japonica*	5	31.00 ± 1.21	1	0	2	0	2	7.33 ± 0.16	2.01 ± 0.206
5	Nua shan	*japonica*	3	30.10 ± 1.11	3	0	2	0	2	6.98 ± 0.31	2.08 ± 0.203
6	Nua pai	*japonica*	3	26.66 ± 0.28	1	0	2	1	2	5.32 ± 0.54	2.13 ± 0.312
7	Mo do	*indica*	1	27.32 ± 0.14	1	0	4	1	1	4.98 ± 0.76	1.98 ± 0.120
8	Khau chung	*indica*	1	30.52 ± 0.28	9	0	3	0	2	5.23 ± 0.09	2.79 ± 0.29
9	Khau cam	*indica*	3	24.78 ± 0.39	9	1	7	0	0	9.74 ± 0.15	1.20 ± 0.203
10	Nep den	*japonica*	3	24.75 ± 0.24	4	0	7	0	1	5.34 ± 0.09	2.13 ± 0.248
11	Chan tom	*japonica*	3	29.45 ± 0.08	0	0	2	1	0	5.40 ± 0.07	2.26 ± 0.13
12	Beo dang	*indica*	1	26.52 ± 0.15	10	0	2	0	0	4.70 ± 0.09	1.56 ± 0.29
13	Khau coi	*japonica*	3	21.28 ± 0.08	1	0	2	1	0	4.12 ± 0.01	1.98 ± 0.408
14	Lua van	*indica*	3	27.32 ± 0.04	3	1	2	1	0	10.96 ± 0.13	1.15 ± 0.07
15	Nep lai te	*indica*	1	29.07 ± 0.24	0	0	4	1	1	12.39 ± 0.43	2.97 ± 0.16
16	Te som	*indica*	1	23.40 ± 0.23	4	0	2	1	0	11.36 ± 0.27	2.23 ± 0.04
17	Lua mo	*indica*	3	21.82 ± 0.25	4	0	4	2	0	9.83 ± 0.53	2.89 ± 0.15
18	Blaute	*indica*	3	30.38 ± 0.47	1	0	1	3	2	13.45 ± 0.69	2.97 ± 0.20
19	Te muon	*indica*	1	25.52 ± 0.12	10	0	2	1	0	11.04 ± 0.45	2.41 ± 0.18
20	Nep hoa vang ray	*japonica*	5	29.13 ± 0.13	1	0	2	2	2	11.73 ± 0.38	2.87 ± 0.09
21	Nep Ha Tuyen to	*indica*	3	29.32 ± 0.10	3	1	2	3	1	11.24 ± 0.27	2.32 ± 0.07
22	Nep trang an	*japonica*	3	28.47 ± 0.14	3	0	2	3	2	10.87 ± 0.22	2.95 ± 0.10
23	Nep Ha Tuyen nhỏ	*indica*	3	25.74 ± 0.20	10	0	2	3	2	11.76 ± 0.57	2.06 ± 0.28
24	Nuong trang	*indica*	1	29.56 ± 0.15	10	0	1	1	0	8.87 ± 0.68	1.91 ± 0.29
25	Lua non	*indica*	1	37.60 ± 0.40	10	0	2	3	2	7.33 ± 0.79	1.69 ± 0.12
26	Nep cam	*indica*	3	29.93 ± 0.09	9	0	7	3	1	9.53 ± 0.69	2.44 ± 0.12
27	Khau ngan	*indica*	3	32.72 ± 0.39	10	0	2	3	0	6.30 ± 0.45	1.95 ± 0.23
28	Cham pet	*indica*	1	24.54 ± 0.13	10	0	2	1	0	6.42 ± 0.45	2.41 ± 0.19
29	Khau giang	*indica*	2	29.17 ± 0.13	4	0	4	1	0	8.41 ± 0.81	2.29 ± 0.15
30	Khau san	*japonica*	2	30.25 ± 0.34	0	0	2	3	2	7.72 ± 0.15	2.54 ± 0.14
31	Cham cau	*indica*	1	30.37 ± 0.50	2	0	2	1	0	7.24 ± 0.47	2.69 ± 0.43
32	Linh chang	*indica*	1	26.66 ± 0.60	3	9	2	1	1	7.11 ± 2.01	1.64 ± 0.16
33	Khau pum	*indica*	2	30.78 ± 0.14	0	0	1	3	2	15.92 ± 1.51	4.23 ± 0.29
34	Lai meo	*japonica*	2	30.69 ± 0.13	9	1	2	1	0	8.08 ± 0.25	2.07 ± 0.16
35	Khau nam	*japonica*	2	24.87 ± 0.27	0	0	1	3	1	10.70 ± 2.38	2.57 ± 0.11
36	Mong chua	*japonica*	2	23.38 ± 0.12	1	0	1	2	1	5.91 ± 0.09	2.82 ± 0.21
37	Pe lenh	*japonica*	2	25.71 ± 0.21	5	1	3	3	2	5.29 ± 0.22	2.66 ± 0.20
38	Khau xay pu	*indica*	1	28.02 ± 0.19	2	1	2	2	2	9.25 ± 0.07	2.67 ± 0.16
39	Nep tan thom	*indica*	2	28.81 ± 0.18	2	5	2	1	1	5.40 ± 0.11	2.56 ± 0.11
40	Te som	*indica*	1	24.24 ± 0.22	4	0	4	3	2	9.12 ± 0.22	4.01 ± 0.20
41	Te meo den	*indica*	1	27.24 ± 0.06	2	9	1	1	1	4.87 ± 0.04	4.75 ± 0.13
42	Buyt ton mau	*indica*	1	24.52 ± 0.22	3	1	2	3	1	7.86 ± 0.07	2.80 ± 0.06
43	Beo ma cu	*japonica*	2	24.89 ± 0.14	5	1	5	3	2	11.61 ± 0.16	2.97 ± 0.07
44	Khau phet	*indica*	1	27.46 ± 0.22	2	2	2	1	0	4.24 ± 0.09	3.45 ± 0.16
45	Khau nua luong	*indica*	1	25.62 ± 0.17	3	0	1	3	1	5.52 ± 0.11	3.65 ± 0.17
46	Khau chat	*indica*	1	27.35 ± 0.08	2	9	1	1	0	10.10 ± 0,05	3.65± 0.17
47	Khau nua tau	*indica*	1	26.71 ± 0.09	1	1	1	2	2	5.08 ± 0.20	3.94 ± 0.19

D. *The genetic diversity of 12 local upland rice cultivars*

We have selected 12 cultivars the 47 cultivars to evaluate the genetic diversity by RAPD technique. The local name and symbol of these cultivars are shown in Table III. The products of RAPD reactions were analyzed via electrophoresis in agarose gel (Fig. 1). All of the primers showed polymorphism, in which, the primer RA45 showed the highest polymorphism (100%) and the primer RA31 amplified minimum number of fragments (50% of polymorphic fragments). The similarity and difference coefficients were determined by using NTSYSpc-2.02i program.

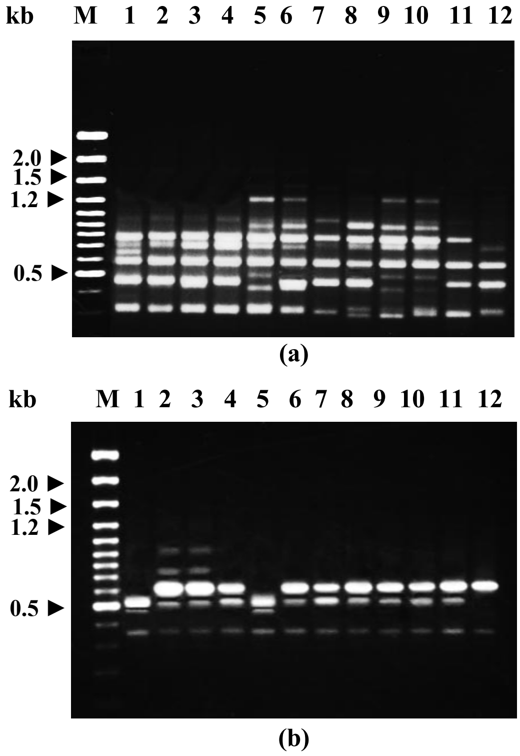

Figure 1. Electrophoresis of RAPD products with primer RA46 (a) and primer RA32 (b) (M: 100 bp DNA ladder; 1- SL2; 2-SL3; 3-SL4; 4-SL5; 5-SL6; 6-SL7; 7-BK9; 8-BK10; 9-BK15; 10-CB16; 11-CB17; 12-CB18)

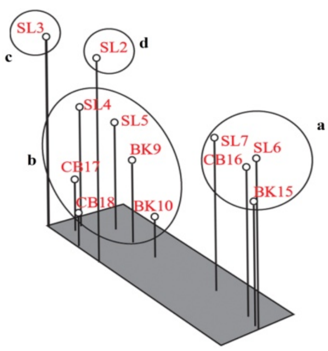

Figure 2. The 3D scatter plot shows the genetic relationship at the DNA level of 12 upland rice cultivars

TABLE III. DROUGHT TOLERANCE INDEX OF 12 UPLAND RICE CULTIVARS

Symbol	Local Name	Drought Tolerance Index
SL2	Mong Chua	16361,42
SL3	Pe Lenh	5459,04
SL4	Khau Xay Pu	14949,62
SL5	Nep Tan Thom	9308,16
SL6	Te Som	17909,52
SL7	Te Meo Den	15282,69
BK9	Buyt Ton Mau	17649,54
BK10	Beeo Ma Cu	14247,09
BK15	Khau Phet	3227,30
CB16	Khau Nua Luong	13782,84
CB17	Khau Chat	18940,70
CB18	Khau Nua Tau	15333,55

The lowest similarity coefficient (48.72%) was obtained between the two cultivars SL3 and SL6, and the highest was obtained between SL5 and BK9 (92,31%). Two couples CB16-SL6 and CB16-BK15 have the lowest difference coefficient (1.12%) and the couple BK9-BK10 has the highest difference coefficient (9.75%). Based on the genetic similarity and difference coefficient, the three dimension scatter plot (Fig. 1) was contructed by using NTSYSpc -2.02i program, showing the genetic relationship at the DNA level of 12 upland rice cultivars. Fig. 1 shows that these cultivars can be divided into 4 groups a, b, c and d. The group a includes 4 cultivars: SL7, BK15, CB16 and SL6 belong to the subspecies *indica*. The genetic distance between the cultivars in group a and those in three other groups is 1.00 – 0.66 = 0.34 (34%). The group d has only cultivar SL2 belongs to the subspecies *japonica*, and the genetic distance between this cultivar and those in groups b and c is 1.00 – 0.67 = 0.33 (33%). The group c also has only cultivar SL3 belongs to the subspecies *japonica*, and the genetic distance between this cultivar and those in groups b is 1.00 – 0.694 = 0.306 (30.6%). The group b consists of 6 cultivars, including 5 cultivars belong to the subspecies *indica* (SL4, SL5, BK9, CB17 and CB18) and the cultivar BK10 belongs to the subspecies *japonica*. The genetic distance between the cultivar SL5 and BK9 is the highest (92.31%). The genetic distances between the cultivars in group b range from 7.69% to 22.40%. The genetic diversity coefficient at the DNA level of these upland rice cultivars is H_g = 52,37%.

E. *The phenotypic diversity for drought tolerance*

Based on the analysis of 21 factors relating to drought tolerance, we have determined the similarity and difference coefficient of the reactivity of pairs of upland rice cultivars by using NTSYSpc - 2.02i program. The dendrogram in Fig. 3 describes the similarity of drought tolerant phenotype of local upland rice cultivars.

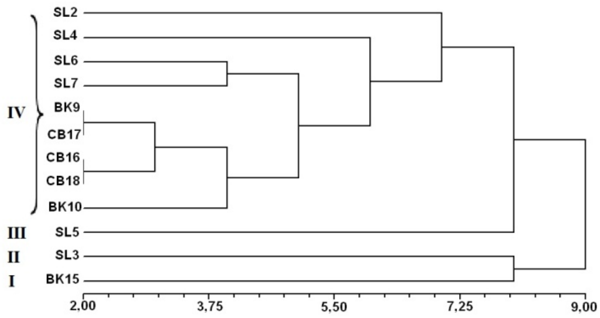

Figure 3. The dendrogram describes the diversity of drought tolerant phenotype of 12 upland rice cultivars

The analytical results showed that the similarity coefficient of the drought reactivity ranged from 0% to 28.571%. The highest similarity coefficient (28.571%) was obtained between these couple of cultivars: CB16-CB17; CB16-CB18; BK9-CB16 and BK9-CB17. The lowest similarity coefficient (0%) was obtained between the cultivar SL3 and the cultivars SL2, SL4, SL5, SL6, SL7, BK9, BK10, BK15, CB16 and CB18; and between BK15 and the cultivars SL2, SL4, SL5, SL6, SL7, BK9, BK10, CB16 and CB17. All the cultivars are different in the reactivity for drought. The difference coefficients range from 1.003% to 9.394%, in which the lowest was obtained between BK9 and SL7 and the highest was obtained between SL4 and SL5.

The relative drought tolerance index ranged from 3227.30 (BK15) to 18940.70 (CB17) as shown in table 1. The dendrogram in fig. 3 showed that, 12 upland rice cultivars were divided into 4 groups with different possibilities of drought tolerance (group I, II, III and IV). Group I includes the cultivar BK15, group II includes SL3, group III includes SL5, group IV includes 9 cultivars, divided into 4 branchs: branch 1 – SL2, branch 2 – SL4, branch 3 includes 2 cultivars SL6 and SL7, branch 4 includes 4 cultivars: CB16, CB17, CB18 and BK10. The diversity coefficient of drought tolerant phenotype is H_{ph} = 73.15%.

IV. CONCLUSION

We have collected and classified 47 local upland rice cultivars in which there are 33 cultivars belong to subspecies *indica* and 14 cultivars belong to subspecies *japonica*. The diversity in morphological and qualitative as well as the protein and lipid content indicated the diversity of these cultivars.

Application of RAPD technique in order to analyse genome of 12 upland rice cultivars and by using the programme NTSYSpc-2.02i , we have determined the genetic similarity and difference coefficient of these cultivars. The dendrogram was constructed with 4 group. The genetic distances between cultivars range from 7.69% to 34.0%. The genetic diversity coefficient of these cultivars is H_g = 52.37%.

The reactivities for drought of these upland rice cultivars were shown in the diversity of drought tolerant phenotype. These cultivars were divided into 4 groups according to the relative drought tolerance index. The difference coefficients range from 1.003% to 9.394% and the phenotypic diversity coefficient H_{ph} = 73.15%.

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