CHAPTER 2

PHENOTYPIC VARIATION IN PURPLE RICE VARIETIES

2.1 Introduction

Rice is rich in genetic diversity, with thousands of varies grown throughout the world (FAO, 2004). The thousands of cultivated varieties of *O. sativa* varied greatly in growth habit, from, size and structure (Chang, 1965). In its natural unpolished state rice comes in many different colors, including brown, red, purple and even black. These colorful rice varieties are often prized for their health properties. Unpolished rice has a higher nutrient content than milled or unpolished white rice (FAO, 2004).

In rice diversity, purple rice is a unique type of having pigmented in various phenology characters. Purple glutinous indica purple rice was cultivated in Thailand and as our ancient wisdom, purple rice was considered to have an herbal property and has been used as a traditional Thai herb for various kinds of a medicinal treatment. Differing among the cultivated varieties depended on the pigmentation of purple color (anthocyanin) on the plant part (Hayashi and Abe, 1952), enriching the colorful agro-biodiversity of Thailand rice field. Since, the domination of the white modern rice varieties in the rice extension program for commercially rice, this local rice type was less attended of sowing. Developmental activities and monoculture of modern varieties were replacing the landraces. The colorful of agro-biodiversity in the rice field has diminished. Cultivation of pigmented rice is unnecessary. The rice diversity in Thailand was limited and the germplasm is in danger.

In this report, experiments were set up to classify the phenotype variation among the rice Thai purple varieties. Agronomic characters such as leaf, stem and grain were analyzed. Results could provide understanding in genetic diversity of the Thai purple rice germplasm. The high range of the variants could benefit breeding programs aiming for upgrading the accumulation level of the nutrients. The Thai local purple rice diversity could prove a priceless heritage of rice germplasm.

2.2 Materials and Methods

Thirteen variety of local glutinous purple rice genotypes, collected in Thailand (KonKean, Chiangmai, Nan, Mae Hongson provinces) were grown at research field of Agronomy Department, Agriculture Faculty, Chiang Mai University.

The characteristics of the adult plant were recorded during or shortly after anthesis (Chang, 1965). Morphological and physiological characteristics were recorded individually using method of IRRI-IBPGR (1980) at differenced stages;

- *Tillering:* color at different plant parts including, leaf blade, leaf sheath, auricle and ligule, and tillers shape.
- *Flowering:* presentation of awn, color of apiculus and stigma.
- *Maturity*: culm length (cm), flag leaf length and width, Flag leaf angle
- *Harvesting*: About ten seeds from each plant were determined for seed width, seed length, whole grain color, husk pubescence, and pericarp color.

One hundred grains in each sample were characterized based on husk color, pericarp color, grain width and grain length. The grain size and shape grain were classified into round, slender, and large grain type (Oka, 1988).

2.3 Result

2.3.1 Leaf sheath and leaf blade color

The leaf sheath was wrapped around the clum above the node. Leaf blades were generally flat and many parallel veins in the upper surface. The underside of the leaf blade was smooth with the prominent ridge in the middle (mid rib). The uppermost leaf below the flag leaf on the main culm was taken as a representative leaf blade. Leaf sheath color was taken on the first leaf below the flag leaf. Leaf blade colors were either full purple or purple margins. Furthermore, leaf sheath colors were either full purple or purple lines. Relation among color of leaf sheath and leaf blade was identified. The leaf sheath with full purple color, its leaf blade is also full purple. Via visa, the purple regime leaf blade and the sheath with purple lines, indicated a similar color of the leaf blade. There was no variation within color in both leaf sheath and leaf blade (Table 2.1) (Figure 2.2 and 2.3).

2.3.2 Auricle and ligule color

Most leaves possessed small, paired ear-like appendages on ether side of the base of the blade. These appendages were called auricles which may not be present on older leaves. Another leaf appendage was the ligule, the papery membrane at the inside juncture between the leaf sheath and the blade. It had either a smooth or hairlike surface. Characteristics of the ligule and the auricle were also recorded on the first leaf below the flag. The length and shape of the ligule differ according to variety. While a ligule of the 13 collections was only purple in color, the color of an auricle exhibited either purple color or colorless (Table 2.1) (Figure 2.4).

2.3.3 Tiller shape, node and internode

Tiller shape was measured from the angle of the tillers. Purple rice varieties were erect in tiller shape (Table 2.1). The node was the solid portion of the clum. Mature internode was hollow, finely grooved with a smooth outer surface. Generally, internodes increased in length from the lower to the upper portion of the plant. The lower internode at the plant base was short and thick. While a color of node presented in yellow or green, internode exhibits mainly a color of purple and purple lines. No relation between node and internode colors was identified. A yellow node could either be purple or purple lines internode and a green one could be only purple internode (Table 2.1) (Figure 2.4).

Table 2.1 The color of leaf blade, leaf sheath, node, internode, auricle, ligule and the shape of ligule and tiller in purple rice

Varieties	Leaf blade	leaf sheath	Node	Internode	Auricle	Ligule	Ligule shape	Tiller shape
Kum 19104	purple strip	purple strip	green	purple	purple	purple	2-Cleft	erect
Kum 19959	full purple	purple strip	green	purple	purple	purple	2-Cleft	erect
Kum 7677	purple margins	purple lines	yellow	purple	purple	purple	2-Cleft	erect
Kum 87061	purple margins	purple lines	yellow	purple	purple	purple	2-Cleft	erect
Kum 88061	purple margins	purple lines	green	purple	purple	purple	2-Cleft	erect
Kum 89038	purple margins	purple lines	green	purple	purple	purple	2-Cleft	erect
Kum 99151	purple margins	purple lines	yellow	purple	purple	purple	2-Cleft	erect
Kum Doi Musur	full purple	full purple	green	purple	purple	purple	2-Cleft	erect
Kum Doi Saket	full purple	full purple	green	purple	purple	purple	2-Cleft	erect
Kum Na	purple strip	purple strip	green	purple	purple	purple	2-Cleft	erect
Kum Nan	full purple	full purple	green	purple	purple	purple	2-Cleft	erect
Kum Omkoi	purple margins	purple lines	yellow	purple lines	purple	purple	2-Cleft	erect
Kum Vengsa	full purple	full purple	yellow	purple	purple	purple	2-Cleft	erect

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2.3.4 Length and width of flag leaf

Rice leaf length and leaf width were remarkable leaf characteristics in the modern rice breeding programs. Leaf length was more associated with high yielding ability than leaf width. In purple rice, there was variation of flag leaf length and width (36.5 and 1.8 cm on average, respectively). The range of length was 24.1 to 48.7 cm Kum Veng Sa had the longest flag leaf but Kum 89057 was the shortest of flag leaf. The range of flag leaf width was 1.4 to 2.2 cm and average was 1.8 cm. Kum Doi Mu Sur was the widest (2.2 cm) however, Kum 89038 was the shortest (1.4 cm) of flag leaf width (Table 2.2).

2.3.5 Ligule length and main stem diameter

Leaf ligules were used as a key identification index of taxonomy in grasses. The ligule length was also used in classification. Although most rice varieties contained ligules, different varieties usually contain various ligule lengths or shapes. The variation was found in ligule length and main stem diameter. The average was 19.3 mm and 0.8 cm respectively (Table 2.2). In Ligule length, Kum Nan was the longest (30 mm) but Kum 87090, Kum Om Koi, Kum 87061, Kum 88083, Kum 88038 and Kum 88069 were the shortest group. The main stem diameter varied from 0.5 to 0.99 cm. The widest was Kum 19959 (1.3 cm) but Kum 89038 was the shortest (0.5 cm). Many collections exhibited a strong main stem (0.83-.099 cm. diameter). While others were a moderate strong main stem. Kum 7677 and Kum 89061 was a weak stem (0.5 cm diameter) (Table 2.2).

Table 2.2 Main stem diameter, ligule length, width and length of flag leaf, whole grain and unpolished grain and length-width ratio

Varieties	Main stem	Ligule length	Flag leaf	Flag leaf (cm)		whole grain (mm)		unpolished grain (mm)		Length/ width	
	diameter (cm)	(mm)	angle	width	length	width	Length	width	Length	whole grain	unpolished grain
Kum 19104	1.0	20	90	1.7	30.0	3.5	9.2	2.6	6.0	2.6	2.3
Kum 19959	1.3	20	90	2.2	43.5	3.6	10.1	2.9	6.6	2.8	2.3
Kum 7677	0.6	15	45	1.5	40.0	3.5	9.6	2.7	6.0	2.8	2.2
Kum 87061	0.7	15	80	1.9	38.1	3.4	8.7	2.6	5.7	2.5	2.2
Kum 88061	0.8	19	80	1.8	36.1	3.4	8.2	2.9	6.1	2.4	2.1
Kum 89038	0.5	15	75	1.4	38.3	3.4	8.6	2.6	6.2	2.6	2.4
Kum 99151	0.7	18	75	1.7	43.8	3.4	8.8	2.8	5.7	2.6	2.0
Kum Doi Musur	0.8	20	90	2.2	35.6	3.5	9.2	3.0	6.3	2.7	2.1
Kum Doi Saket	0.9	20	80	1.9	44.5	3.4	9.8	2.8	6.6	2.8	2.4
Kum Na	1.0	20	90	1.9	28.2	3.5	9.1	3.1	6.0	2.6	1.9
Kum Nan	1.0	30	90	1.8	32.7	3.5	9.9	2.7	6.3	2.8	2.3
Kum Omkoi	0.9	15	80	1.8	34.6	3.2	8.8	2.8	5.9	2.8	2.1
Kum Vengsa	0.8	20	90	2.0	48.7	3.5	9.5	2.8	6.3	2.7	2.3
Mean	**0.8**	**19.0**	**81.2**	**1.8**	**38.0**	**3.4**	**9.2**	**2.8**	**6.1**	**2.7**	**2.2**
SD	**0.2**	**4.0**	**12.4**	**0.2**	**6.0**	**0.1**	**0.6**	**0.2**	**0.3**	**0.1**	**0.1**

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2.3.6 Seed length

In whole grain, seed length varied from 8.2 – 10.1 mm. The average of seed length in 13 purple rice genotypes was 9.2 mm. Kum 19959 was the longest and Kum 88061 was the shortest. In unpolished grain, seed length varied from 5.7 – 6.6 mm and the average was 6.1 mm. Kum 19959 also presented the longest and the shortest was Kum 87061 and Kum 99151 (Table 2.2).

2.3.7 Seed width

There were variations of seed width in both whole grain and unpolished grain. In whole grain, the variation ranged from 3.2 – 3.6 mm and average was 3.4 mm. Kum 19959 was the widest and Kum Omkoi was the narrowest. In the unpolished grain, seed width varied from 2.6 – 3.1 mm and average was 2.8 mm. Kum 19104 was the narrowest and Kum Doi Saket was the widest of seed width (Table 2.2).

2.3.8 Seed shape

Seed shape variation based on seed length and width ratio to classify unpolished grain into round, slender, and large grain type (Watabe, 1967) was used. All purple rice samples were classified into round grain (Figure 2.1).

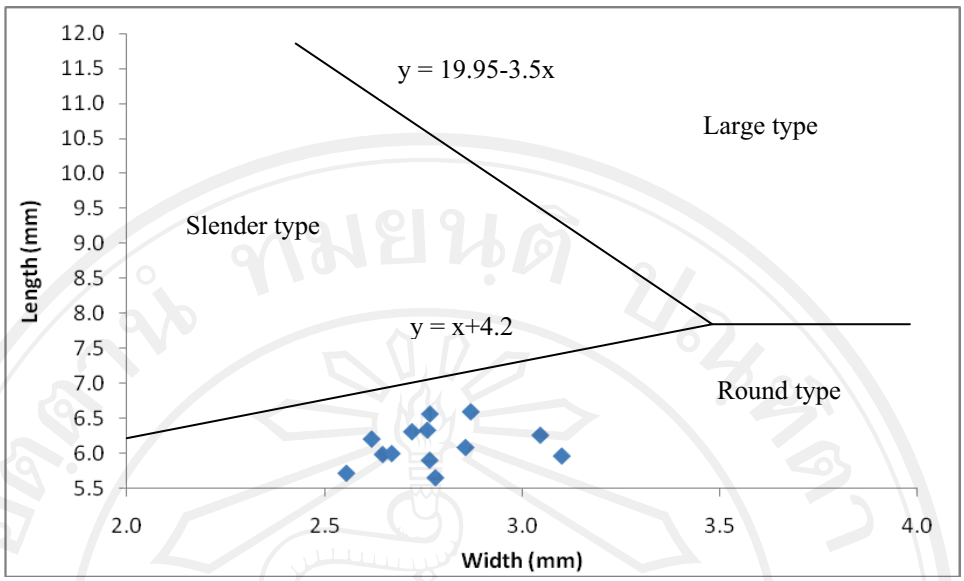

Figure 2.1 Grain shape of unpolished grain in purple rice collection

2.3.9 Stigma, Glumes, Apiculus and Awn

Stigma color was either purple or colorless (Figure 2.6). The apiculus and glumes of the 13 varieties collected, were purple, purple red and brown in color. Collection Kum 88061 which leaf sheath and blade were green, its glumes and apiculus were in similar color of purple and dark purple as others. All varieties collected were awnless (Table 2.3).

2.3.10 Grain Hull and Pericarp

Similar to a color of an apiculus, a grain pericarp of a purple rice genotype would only be purple in color. Grains of the 13 collections were all purple colored of a pericarp either purple or dark purple. This indicated that a purple color of a grain pericarp is a criterion to identify a purple rice genotype. And further, a purple pericarp of rice is a major part of plant to accumulate an anthocyanin. A varied in

purple color of a pericarp may also indicate a varied in an amount of the anthocyanin and also γ-oryzanol accumulated (Table 2.3) (Figure 2.7).

Table 2.3 The color of stigma, apiculus, awn, glume, pericarp and panicle shape in purple rice collection

Varieties	Stigma	Apiculus	Awn	Glume	Pericarp	Panicle shape
Kum 19104	purple	purple	awnless	purple	purple	very pack
Kum 19959	purple	purple	awnless	purple	purple	very pack
Kum 7677	purple	purple	awnless	purple	purple	pack
Kum 87061	purple	purple	awnless	dark purple	dark purple	spread
Kum 88061	purple	purple	awnless	dark purple	dark purple	pack
Kum 89038	purple	brown	awnless	purple	dark purple	pack
Kum 99151	purple	purple	awnless	purple	purple	very pack
Kum Doi Musur	purple	purple red	awnless	purple	purple	pack
Kum Doi Saket	purple	purple red	awnless	purple	purple	pack
Kum Na	colorless	purple	awnless	purple	purple	very pack
Kum Nan	purple	purple red	awnless	purple	purple	pack
Kum Omkoi	colorless	brown	awnless	purple	purple	spread
Kum Vengsa	purple	purple red	awnless	purple	purple	very pack

2.4 Discussion

In any plant breeding program, genetic diversity is essential, by providing the raw material for hybridization. Without this material diversity, crop improvement is almost impossible. The reduction in biodiversity often increases in vulnerability to stress changes such as climate and pathogens. Further, may raise a risk for individual farmer and can undermine the stability of agriculture (WRI, 2002). The wide range of

diversity means a better choice for a breeder to select for the appropriate kind of variants and that a better chance for his success.

In many years past, plant breeders have trying to produce high yielding varieties. As a result, a production of many crops relies heavily on a few modern varieties which is very uniform and contains less genetic diversity than land race varieties. Mono culture of uniform varieties has shown a serious economic loss. Homogenization of varieties increases vulnerability to insect pests and diseases (Schoenly *et al.,* 1998). Comparison between rice varieties planted in mixtures and in mono cultures in Yunan province of China showed the rice planted in mixture had 89% greater yield, blast disease was 94% less severe than rice planted as mono cultures (Zhu *et al.,* 2000).

Rice diversity had been reviewed by many researchers. Dilday *et al*. (1998) evaluated within 17,279 accession had found that grain length ranged from 3.0 to 9.9 mm. with the length and width ratio ranging from 1.0 to 8.0, 1000-grain weight ranged from 6.9 g to 40 g Mounmeuangxam (2003) evaluated rice varieties collected in Houaphanh province in Lao PRD found a grain length and width ranged from 6.41 to 9.92 mm and 2.83 to 4.06 mm respectively, 1000-grain weight ranged from 24 to 41 g The minimum values of grain size of length and width of these two authors may differ but the maximum values were about equal.

In my study, 13 accessions of purple glutinous rice were collected in the north and north eastern Thailand in which the rice was produced to meet a household need rather than for sale. It was found that the color of leaf sheath and leaf blade varied from purple line to purple. Moreover, there was no variation within color in both leaf sheath and leaf blade. In addition, the range of flag leaf length was 24.1 to 48.7 cm

and flag leaf width was 1.4 10 2.2 cm. The result was the same in modern oriental rice cultivars in U.S. which were a wide range of variation in the two flag characteristics (Kuo and Li, 1994).

Morphological features of leaf ligules were used as a key identification index of taxonomy in grasses. For example cereal crops such as rice maize and wheat, usually possessed a ligule while barnyard grasses do not (Josef, 1972). Although most rice varieties contained ligules, different varieties usually contained various ligule lengths or shapes. The range of ligule length of double haploid rice population varied from 5.8 mm to 17.3 mm (Dali *et al.,* 2007). In this study, the legule length differed according to variety. The range of ligule length of purple rice in this collection was 15 mm to 30 mm. Furthermore, the ligule of the 13 collections was only purple in color, and the color of an auricle exhibited either purple color or colorless.

Stigma color was either purple or colorless. These findings agreed with the morphology studied conducted by Elizabeth *et al* (2008) indicating that, stigma color varied among and within *O. glumaepatula* populations which present white and purple stigma. In addition, Syed *et al* (2007) found the variation of stigma color in rice CMS lines that varied from white, lower half white to purple. It may indicate that purple rice was land race which had wild rice ancestor and had been cultivated that are adapted to varied environmental condition.

Figure 2.2 Variation of leaf sheath color in purple rice varieties

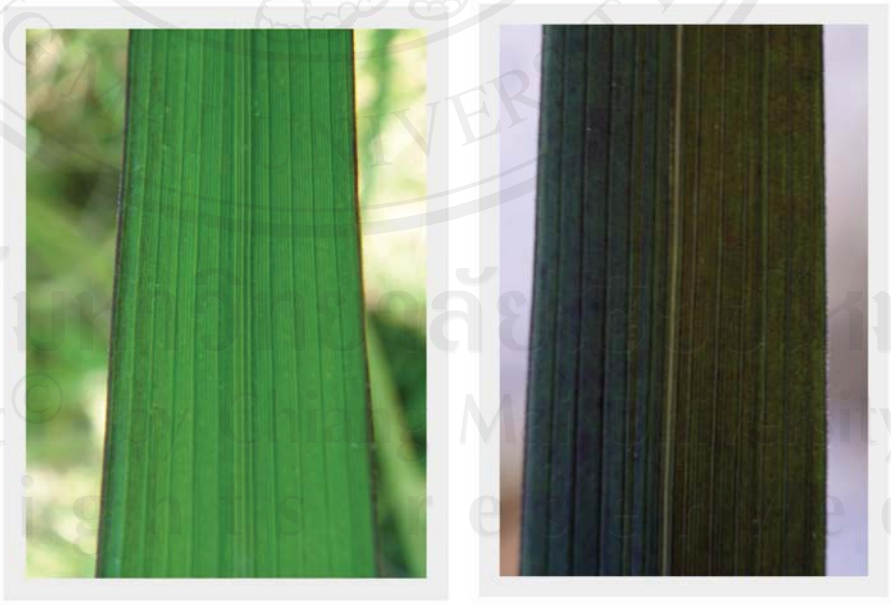

Figure 2.3 Variation of leaf blade color in purple rice varieties

Figure 2.4 Variation of node and inter node color in purple rice varieties

Figure 2.5 Variation of auricle and ligule color in purple rice varieties

Figure 2.6 Color variations in purple rice varieties

Figure 2.7 Color variations of husk and pericarp in purple rice varieties