MORPHOLOGY AND AGRONOMIC TRAITS OF EIGHT CAMBODIA RICE LANDRACES

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ABSTRACT

Rice, a staple crop of Cambodia, is mostly cultivated in rain-fed lowland and upland, accounting for about 85 % of the cropping areas. Rice landraces in Kampong Thom province are maintained and cultivated by farmers for specific needs and conditions of individual farming systems and are likely sources of germplasm for breeding new rice varieties. The field survey found the total of eight landrace varieties including Neangkert (NK), Laksleuk (LS), Kolphaav (KP), Phrech (PR), Kromounsor (KMS), Domnerbses (DS), Chaorng (CH) and Neangkong (Nkong) from Sandan and Prasatsombor district. Eight landrace varieties are well recognized for significant traits like aroma, taste and disease resistance. The aim of this study was to examine morphological diversity of rice landraces germplasm grown in two districts, Sandan and Prasatsombor, Kampong Thom province. Data were recorded for traits such as kernel (length, width and thickness), and seed coat (length, width, and thickness), plant height, and tiller number. The results showed that there were significant differences (P<0.05) among the eight traditional rice varieties in morphological characters e.g. length, breadth and thickness of seed and seed coat. The data representing the length of unhulled and hulled rice seeds showed that Domnerbses (DS) verity had the longest seed significantly. Moreover, the Chaorng (CH) variety had the highest breadth and the thickest seed and seed coat. The result found that CH variety had the highest height of rice. Meanwhile, other seven rice landraces had quite similar trend of growing. For tiller

number, each of rice variety had totally different number of tiller. Among eight rice varieties, PR variety tended to have higher number of tiller number than others. For quantitative and qualitative of diversity, overall, leaf color of eight rice landraces was the highest diversity index by KP was the highest leaf color diversity among eight varieties. Nkong and CH were found for the highest seed diversity index of seed color, which were 1.31 and 1.38, respectively. KP, KMS, NK and PR were found in the highest seed H' of seed length ratio, which were 0.64, 0.24, 0.5 and 0.67, respectively. The result of correlation analysis showed that, there were positively and highly significant correlation between 1000-grain weights with grain width (r= 0.88), grain thickness (r=0.88), seed coat width (r=0.86), seed coat thickness (r=0.83), length of panicle (r=0.38), harvesting time (r=0.78) and tiller per plant (r=0.51) and negatively correlation with tiller number (r= -0.34). The characters had positive relationship with 1000-grain weight were plant height (r=0.31), grain length (r=0.03). Among these characters only seed coat length (r=-0.06), seed per panicle (r=-0.07) were non-significant correlation. Plant height had positive significant correlations with all characters except harvesting time, tiller per plant, 1000-grain weight and weight. At the same time, grain length and seed coat length had negatively a significant correlation. Among these character only tiller number showed negative non-significant correlation with plant height. Significantly negative correlations were shown for tiller number with grain width, grain thickness, seed coat width, harvesting time, tiller per panicle and 1000-grain weight which were r = - 0.36, r -0.34, r = - 0.40, r= - 0.32, r = - 0.23, and r = - 0.34, respectively. However, positively non-significant correlation of tiller number were found with grain length, seed coat length which were r = 0.15, r = 0.19, respectively. The principal component analysis (PCA) among 16 morphological traits in the rice varieties for yield contributing characters had a 70.6 % cumulative explained variance of the first two components. The PC1 and PC2 explained 45.2% and 25.4% of the variance, respectively. Future more collection trips and characterization studies would further enrich diversity, in particular traits low diversity, such as anthocyanin coloration, awn presence, awn color, culm habit, panicle type and cooking.

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CHAPTER I

INTRODUCTION

Background

Rice, a staple crop in Cambodia, is mostly cultivated in rainfed lowland and upland, accounting for about 85 percent of the Cambodian cropped area (Man, S., 2007). Khmer farmers have been growing rice for at least 2,000 years, or even longer in the case of upland rice. Historians believe that rice-growing technologies may have been adopted in Cambodia along trading routes from India. Later, irrigated rice production technologies were introduced since 1,500 years ago (Chandler, D. P., 1993; Sokunthea, S., 2004). Farmer integrated rice production into their existing systems of land use which had been developed since prehistoric times. As already stated, rice is the most important agricultural product of Cambodia, contributing about 10 percent to Gross Domestic Product (GDP) of the country (Structural policy country notes Cambodia, 2014). Rice in Cambodia is grown in four different ecosystems, which are, rainfed lowland, rainfed upland, deep water, and irrigated rice (Man, S., 2007). The rice cultivation area has been expanded for 2.8 million ha in 2010 (Maclean, J. et al., 2013). More than 80 percent of rainfed lowland rice is under rice landraces (Javier, E. L., 1997). Therefore, the rice landraces are a very important crop to Cambodian farmers (Man, S., 2007; Chandler, D. P. et al. 2008).

The rice landrace varieties maintained and cultivated by farmers are likely to be the sources of germplasms for the next planning season and the breeding for new rice varieties (Rabara, R. C. et al., 2014). Furthermore, local varieties are generally considered as a rich source of genetic variation. Farmers continue to use the traditional rice varieties because of their grain qualities, medicinal properties, nutrition, taste, aroma, tolerance to drought and submergence and adaptability to abiotic and biotic stresses (Hanamaratti, N. G. et al., 2008). The name of each rice landraces named by farmers describing the phenotypic characteristics of panicle and grains were inconsistent indicators of genetic diversity (Bajracharya, J. et al., 2006). Cambodia rice landraces played an important role in the rice exports of the country before the civil war during the 1970's. After the war, many landrace rice varieties were adapted into different ecosystems. The long civil war forced the rice growing population to move from one place to another, also the import of modern high-yielding varieties was replacing the traditional varieties. The landrace rice varieties in Kampong Thom province are still being used by the farmers because they are well adapted to the local areas. The field survey in Kampong Thom province found that a total of eight landrace rice varieties including Neangkert (NK), Laksleuk (LS), Kolphaav (KP), Phrech (PR), Kromounsor (KMS), Domnerbses (DS), Chaorng (CH), and Neangkong (Nkong) were observed in Sandan and Prasatsombor districts. These eight landrace rice varieties are well recognized for their significant traits like aroma, taste and disease resistance, few studies, however, measured a seed morphological trait of these rice varieties. Therefore, the study of morphological and agronomic characteristics of eight landrace rice varieties from these two districts (Sandan and Prasatsombor districts) in Kampong Thom province could provide the necessary information for plant breeders to improve productivity and quality.

Objective of Study

To examine morphological and agronomic characteristics of the eight landrace rice varieties

Scope of Study

Seed morphology and agronomic traits in eight landrace rice varieties were studied. The samples were collected from two districts (Sandan and Prasatsombo district) in Kampong Thom province, Cambodia. An evaluation of the eight landrace rice varieties was carried out to determine different agronomic and morphological parameters representing quantitative and qualitative data. Trait selection and measurement techniques were based on the IRRI standard evaluation system of rice (IRRI, 2002). All recorded agro-morphological traits were analyzed using two complementary procedures; cluster analysis and principal component analysis (PCA). An analysis of variance (ANOVA) procedure was used to assess the significance of agro-morphological variation among eight landrace rice varieties. All statistical tests were performed using the R program version 3.3.1 (The R Foundation, 2016).

Significance of the study

Neangkert (NK), Laksleuk (LS), Kolphaav (KP), Phrech (PR), Kromounsor (KMS), Domnerbses (DS), Chaorng (CH) and Neangkong (Nkong) are well recognized for their significant traits like aroma, taste and disease resistance from Sandan and Prasatsombor districts in Kampong Thom province. Therefore, the study of morphological and agronomic characteristics of these rice varieties could provide plant breeders with the information necessary to improve productivity and quality.

Terminology

1. **Landrace rice variety** refers to the rice varieties originating in the target environment (an ancients type of rice) and cultivated by farmers, which are likely sources of germplasm. Most of the traditional rice varieties are highly photoperiod-sensitive, with flowering taking place as a response to short giving growth duration from 180 over 300 days. (Rabara, R. C. et al., 2014; Sokunthea, S., 2004).

2. **Morphological characteristic** refers to the shape of the structure on growth and all of the breeding characters include the roots, stems, leaves, flowers and seeds, etc. (Chang, T. T., & Bardenas, E.,1965)

3. **Agronomic characteristic** refers to unique features considered including tillering, seed quality, product, threshing, seeds of the fall, and an adaptation to the environment (Chang, T. T., & Bardenas, E., 1965)

Hypothesis of Study

We hypothesized that the quantitative and qualitative traits such as grain length, width, thickness, plant height, number of tillers per plant, panicle length and 1000 grain weight could be used to describe the agro-morphological variability between these eight landrace rice varieties.

CHAPTER II

LITERATURE REVIEW

Botany, classification, and taxonomy of the rice plant

Cultivated rice (*Oryza sativa* L.) is one of the three major food crops in the world and feeds more than half of the human population. The O.*sativa* is a diploid species having 24 chromosomes, with a basic number of 12 (n = 12 or 2n = 24). However, there are a small number of tetraploid chromosome species (2n = 48) e.g. O. *alta*, O. *grandiglumis*, O. *linggiglumis*, O. *minuta*, O. *punctata*, and O. *ridleyi* (Man, S., 2007). Rice belongs to the genus *Oryza* and the tribe of *Oryzeae* of the family Gramineae (Poaceae). The genus *Oryza* contains approximately 25 species out of which only two species viz. *Oryza sativa (*Asian rice*)* and *Oryza glaberrima (*African rice*)* are recently cultivated (Tsunoda, & Takahashi, 2012). *Oryza glaberrima* is grown only in West Africa countries, whereas *Oryza sativa* is commonly grown in Asian countries and all regions around the world (Khush, 1997). The genus *Oryza* originated about 200-600 million years ago in Gondwanaland (Dudal, & Batisse, 1978). The cultivated species are originated from a common ancestor with AA genome. Perennial and annual ancestor of O. *sativa* is O. *rufipogon* and ancestors of O. *glaberrima* are O.*longistaminata*, and O. *barthii* (Chang, T. T., 1976). The origin of Asian rice cultivation areas (*Oryza sativa* L.) was in India, Bangladesh, and Southeast Asia. Nowadays, in most of the Asia countries, the majority of all rice produced comes from China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Pakistan, Philippines, Korea and Japan. Asian farmers account for more than 80% of the world's total rice production.

Kingdom	Plantae
Division	Anthophota
Order	Graminales
Family	Gramineae or Poaceae
Genus	*Oryza*
Species	*Oryza sativa*

Figure 1 The botanical of rice plant

Source: http://www.wingbeauty.com/store/product/view/oryzanol

In Asia, *Oryza sativa* species are differentiated into three subspecies depend on geographical condition, viz., japonica, javanica and indica. These subspecies can be distinguished by few key characteristics such as glume size, the number of secondary panicle branches, panicle thickness etc. Their characteristics are listed in Table 1.

1. Japonica Rice

Japonica is one of the two main races of *O. sativa japonica*. It is a cluster of rice varieties from northern and eastern China which is grown widely in some areas in the world. It is found in the cool zones of subtropics and in the temperate zones. It has narrow, dark green leaves, medium-height tillers and short to intermediate plant stature. Japonica has several characteristics such as short and roundish grains, awnless to long-awned spikelet, moreover, the grains do not shatter easily and have 0-20% amylose content (Akita, S., 1989)

2. Javanica

Javanica consists of the bulu and gundil varieties of Indonesia. Javanica is a rice variety with broad, stiff, light green leaves. It is low-tillering and it has a tall stature. It's grain is long, broad, and thick. Especially, it does not break easily, and it has 0-25% amylose content. Spikelets are awned or awnless (Akita, S., 1989)

3. Indica

Indica rice is another race apart from the two main races of *Oryza sativa.* It is the major type of rice grown in the tropics and subtropics area. They are mostly cultivated in the Philippines, India, Pakistan, Java, Sri Lanka, Indonesia, Central and Southern China, as well as African countries. Indica's grains are long to short, slender, slightly flat, and the spikelets are awnless. They shatter more easily and have 23-31% amylose content (Akita, S., 1989)

Table 1 Characteristics of Oryza sativa subspecies

Characteristics	Subspecies		
	Indica	**Japonica**	**Javanica**
Tillering	High	Low	Low
Height	Tall	Medium	Tall
Lodging	Easily	Not easily	Not easily
Photoperiod	Sensitive	Non-sensitive	Non-sensitive
Cool temperature	Sensitive	Tolerant	Tolerant
Grain shattering	Easily	Not easily	Not easily
Grain type	Long to medium	Short and round	Large and bold
Grain texture	Non-sticky	Sticky	Intermediate

Source: Bardenas, E. A., & Chang, T. T., 1965

Growth stages of the rice plant

Rice plants usually take 3–6 months from germination to maturity, depending on the variety and the environment under which they are grown. Rice plant growth can be divided into three agronomic phases of development: vegetative, reproductive and ripening phases. The vegetative stage refers to a period from germination to the initiation of panicle primordia, and the reproductive stage is from panicle primordia initiation to heading. Moreover, the ripening period is from heading to maturity. In a tropical environment, rice plant spends about 60 days in vegetative phase, 30 days in the reproductive phase, and 30 days in the ripening phase. The vegetative stage is characterized by the development of tillers, the gradual increase in plant height, and more leaves. Tillering may start when the main culm develops the 5^{th} or 6^{th} leaf. Active tillering refers to a stage when the tiller number per unit of time rapidly increases until the maximum tiller number stage. The maximum tiller number stage is a stage when tiller number per plant or per square meter is maximum. Normally, tiller number declines after the maximum tiller number stage. There is a period before the end stage of effective tillering when the tiller number becomes numerically equal to panicle number at maturity. The reproductive stage is subdivided into preheading and postheading periods. This growth stage is characterized by the increase of plant height, the decline in tiller number, the emergence of the flag leaf, booting, heading, and flowering. Initiation of panicle primordia usually dates back to about 30 days before heading. The beginning of this phase is sometimes referred to the internode elongation that varies slightly by cultivar and weather conditions. The ripening phase starts at flowering and ends when the rice plant is ready to be harvested. Ripening follows fertilization and can be subdivided into milky, dough, yellow, ripe, and maturity stages. These terms are primarily based on the texture and color of the growing grains. The length of ripening varies among varieties from about 15 to 40 days. Ripening is also affected by temperature, with a range from about 30 days in the tropics to 65 days in cool temperate regions (Zakaria, S. et al., 2002).

Rice morphology

The rice plant normally grows for only a year and then dies with round, hollow, stems, flat leaves, and terminal panicle. Rice is cultivated cereal plant adapted to growing in both flooded and non-flooded soils. The rice landraces have the different morphological performances related to the variety and the environment under which they are grown. The morphological performances of rice landrace genotypes clearly refer to a high genetic variation of the local rice. The characterized genotypes may provide an improved crop that resistant to environmental stress in the future (Efendi, et al., 2015). The morphology of rice is divided into the vegetative organs (including roots, culms, and leaves) and the reproductive organs (including panicle, spikelet, flower and grain) (Bardenas, E. A., & Chang, T. T., 1965).

1. **Roots** are fibrous and include rootless and root hairs. The embryonic roots are sparsely branched and persist only for a short time after germination. The secondary adventitious roots are produced from the underground nodes of the young culms, freely branched. As the plant growth, coarse adventitious prop roots are often formed in whorls from the nodes above ground level. Some of the adventitious roots are positively geotropic, while other may be diageo tropic. In floating varieties, fine branched roots are formed from the higher nodes on the long culm below the water surface (Bardenas, E. A., & Chang, T. T., 1965) and root system is a vital of plant and regulates many of shoot growth and development (Yang, J.-c., & Zhang, H., 2012) and rice root growth, and nutrient uptake as influenced by organic manure in continuously and alternately flooded paddy soils systems play an important role in uptake of water and nutrients from soil (Yang, C. et al., 2004). Root morphology and physiology are closely associated with the growth and development of above ground, (Bazzaz, F. A. et al., 2000 as cited in Yang, J.-c., & Zhang, H., 2012).

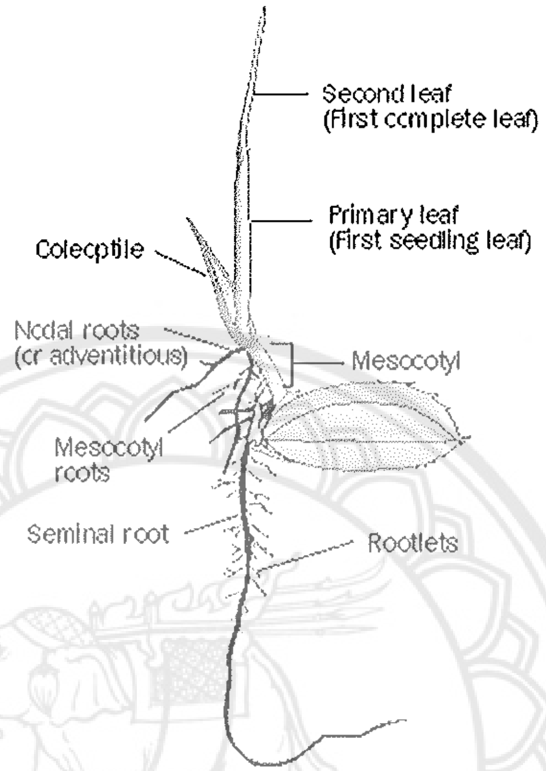

Figure 2 The part of a young seedling germination

Source: Bardenas, E. A., & Chang, T. T. , 1965

2. Stem (Culm): Stem is to support the leaves and reproductive organs. Stem is made up of a series of node and internodes. The node bears a leaf and a bud. The bud is internode in the axil between the nodal septum upper ones. Young internode is smooth and solid. The mature internode is hollow and finely grooved with a smooth outer surface. Normally, the large stem trait is important agronomical characteristics of the rice plant (Wu, et al., 2011).

3. Tiller: The tillering stage starts with the appearance of the primary tillers originate from the axillary bud in lowermost nodes. Afterwards, primary tillers give rise to secondary tillers, and give rise to the tertiary tillers. The number of nodes varies from 13 to 16 with only the upper 4 or 5 separated by long internodes (Bardenas, E. A., & Chang, T. T., 1965).

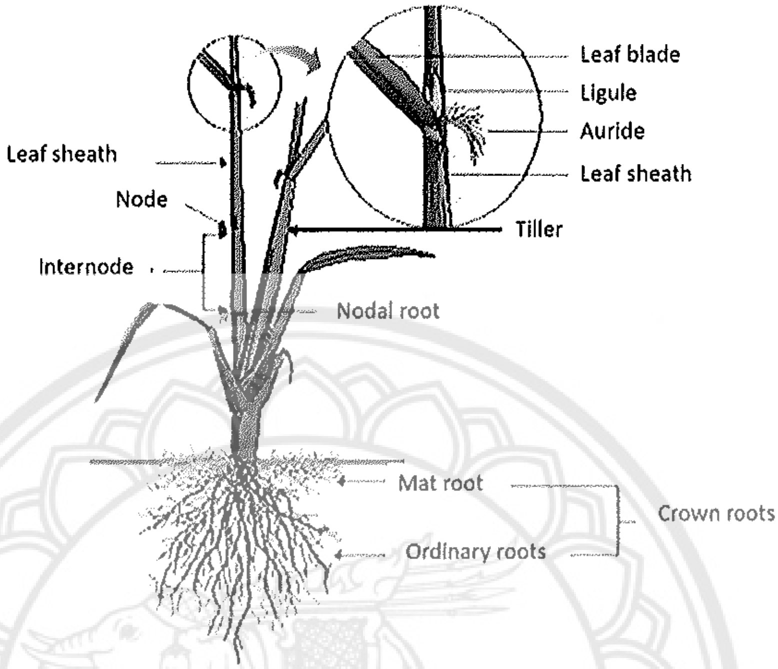

Figure 3 Parts of a primary tiller and its secondary tiller

Source: http://www.ikisan.com/ap-rice-morphology.html

4. **Leaves** are alternately borne on the culm in opposite directions. One leaf is produced at each node. Varieties differ in the number of leaves produced (Figure 3). The leaf consists of the sheath and blade. The leaf sheaths are continuous with the blade. It envelops the culm above the node in varying length, form, and tightness. A swelling at the base of the leaf sheath just above the point of its insertion on the culm is the sheath pulvinus. The sheath pulvinus is usually above the nodal septum and it is frequently mistermed the node. The blades are generally flat and sessile, and they are attached to the node by the leaf sheath. The rice varieties differ in blade length, width, area, shape, color, angle, and pubescence. The uppermost leaf below the panicle is the flag leaf, and it contributes largely to the filling of grains because it supplies photosynthetic products mainly to the panicle. The flag leaf of rice varieties generally differs from the other in shape, size, leaf number and angle. Auricles are small, paired, ear-like appendages borne on either side of the base of the blade. The junction of the blade and sheath on the inside is a membranous, glabrous or ciliate ligule. The ligule varies in length, color, and shape

from variety to variety. The junction of the sheath and blade is the collar or juncture. The collar often appears as a raised region on the back of the leaf. The sheath pulvinus, auricles, ligule and collar on the same plant may be differentially pigmented. Both auricles and ligules are often helpful in differentiating between rice and grassy weeds, which can have auricles or a ligule but not both (Man, S., 2007; Bardenas, E. A., & Chang, T. T., 1965).

5. Panicles and spikelets: The panicles are borne atop the uppermost node on the stem. It is divided into primary, secondary, and sometimes tertiary branches bearing the spikelets. The branches may be arranged singly or in pairs. The panicles stand erect at blooming, but they usually drop as the spikelets fill, mature, and develop into grains. The number of spikelets on panicle, the length, shape, and angle of the primary branches, as well as in the weight of the overall panicle are different considerably with rice varieties. Each individual spikelet contains a set of floral parts flanked by the lemma and palea. The flower consists of six stamens and a pistil. The flower proper consists of the stamens and pistil. The six stamens are composed of 2-celled anthers borne on slender filaments. The pistil contains one ovule. The short style bears the bifurcate, plumose stigma (Bardenas, E. A., & Chang, T. T., 1965). During reproduction, the stigma catches pollen from the stamens and conducts it down to the ovary, where it comes into contact with the ovule and fertilization occurs.

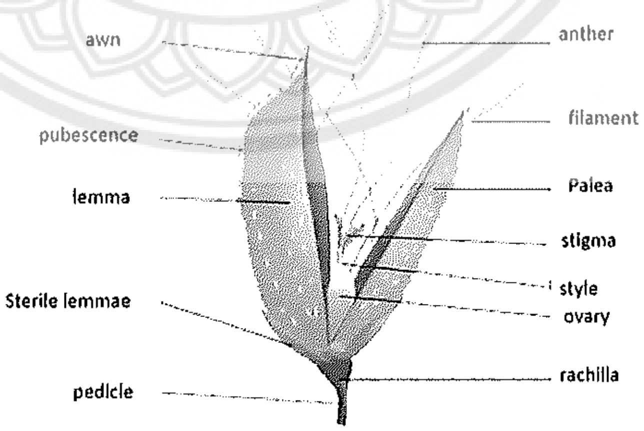

Figure 4 The flowers of rice

Source: modified from http://www.brrd.in.th

6. Seeds: The rice grain, commonly called a seed, consists of the ripened ovary, the lemma and palea, the rachilla, the sterile lemmas, and the awn (not always present). The rice hull includes lemma and palea and their associated structures. Brown rice grain is called caryopsis because of three brownish pericarp layers that envelope it. Beside the pericarp layers are the two tegmen layers and the aleurone layers. The endosperm and the embryo are the remaining part of rice grain. The embryo lies at the ventral side of the spikelet next to the lemma and contains the embryonic root. The rest of the grain consists of endosperm, containing starch, proteins, sugar, fats, crude fiber, and inorganic matter (Bardenas, E. A., & Chang, T. T., 1965). For the traditional rice varieties, normally they produce yield in low to moderate level, but yield has a good characteristic such as cooking characteristics, color, shape, taste and aroma. The average weight of 100 grains is about 2-3 grams or up to 4 grams for some species (Bardenas, E. A., & Chang, T. T., 1965).

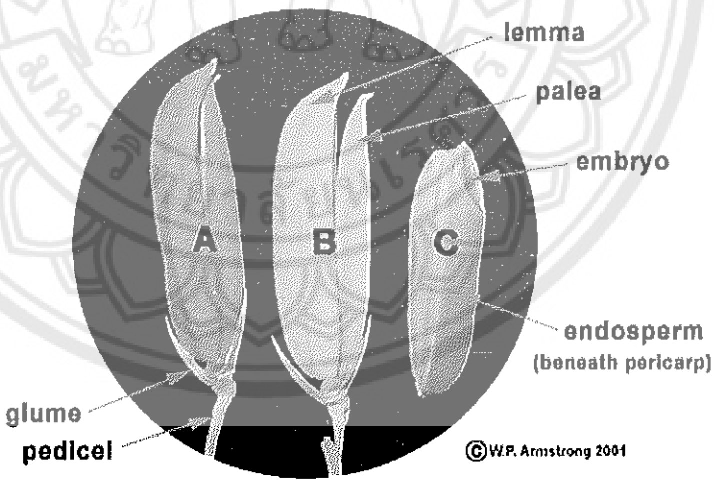

Figure 5 The part of seed rice

Source: modified from http://waynesword.palomar.edu/ecoph12.htm

The importance of rice landraces

Rice landraces are the rice varieties originated in the target environment (an ancient type of rice). Later on, the varieties have been developed by farmers through artificial selection during the long-term domestication process (Ray, A. et al., 2013). Landraces are the traditional varieties of rice, grown by local farmers, which are passed down from generation to generation. Most of landraces varieties are highly photoperiod-sensitive with flowering period as a response to short day growth duration from 180 to over 300 days. Also, these varieties can grow on rainy season and dry season (Kumar, S., Bisht, I. S., & Bhat, K. V., 2010). Landrace rice varieties can be split into many groups since they are diverse in many aspects and the name also varies in each location (Harlan, J. R., 1992). Farmers use morphology to distinct and make it easy to recognize. The morphological characteristic is used to name the rice varieties that look different. Sometimes, the different names of rice come from the same species (Kumar, S., Bisht, I. S., & Bhat, K. V., 2010). This kind of rice has the potential to adapt to local field conditions, unsuitable environmental conditions, and it is resistance to diseases and insects. Moreover, rice landraces can grow in almost any soil types (Khon, 2006 as cited in Man, S. (2007). A period of maturation is different among the varieties (Harlan, J. R., 1992). The rice landraces have a heterogeneous genetic source and high genetic diversity of rice varieties rather than modern varieties (Oka, H. I., 1988). Rice landraces can be a potential source of rice genetics that can serve as a resource for future rice improvement. Rice landrace varieties are diverse in the morphological such as shape, width, grain of length, color of seed, color of leaves, tongue, leaves and the color of stems (Nantiya, P. & Kansamaporn, T., 2011). Landrace rice varieties are well recognized for significant traits like aroma, taste and disease resistance. This rice can be an alternative to farmers who cannot grow improved rice varieties or modern rice (Nantiya, P., & Vijittra, A, 2011). In southern part of Thailand, rice landraces have been observed in 50 varieties (Sangsiri, C. et al, 2012). Moreover, in Cambodia, farmers still grow the landrace rice varieties (Chandler, D. P., 1993; Man, S., 2007; Sokunthea, , S. 2004). There are many types of rice landraces in Cambodia that cannot be described them all. These several examples for landrace rice varieties that are evaluated in term of rice aroma (Man, S., 2007) as following: Ang-Krong, Bonk-Kouy, Neang-Pich, Phka-

Chann, Phka-Chaa,Sombok-Angkrong Donnerb-Krohorm, Dam-Neub-Khao, Dam-Neub-Kror-Chork-Ses, Kror-Moun-Sor, Neang-Mom, Neang-Um, So-Ma-Ly, Mean-Chey, Neang-Sor, Phka-Ma-Lis Kna Phally, Neang-Noun,Kror-Horm, Kong-Kom-Bot,Kror-Ya,Kon-Khmum, Dam-Neub-Tea,Rich, Chom-reun-Phol, Chma-Prum, and all of them are cultivated in rainfed lowland rice areas. While the international rice research institute (IRRI) has about 83,000 accessions, about 9700 or 12% accessions of rice in IRRI have a potential to use these genes for tolerance of drought, cold, deep water, and adverse soil, and resistance to disease, insects, and nematodes (Oka, H. I.,1991).

Morphology and agronomic traits of rice landraces

Rice landraces have involved the detailed observation and recording of morphology and agronomic characterizations and these approaches have served the cause of variety identification. Landraces rice varieties are important plant as genetic resources with potential genetic variation that can be useful for plant breeding programs (Jamago, J. M., & Cortes, R. V., 2012). Moreover, morphological characterization of rice landraces e.g. grain length, grain width, weight of panicle and leaf length have the most substantial contribution (Ray et al., 2013).

Wu et al., (2011) indicated that the systematic modification of morphological, anatomy and physiology was involved in development of the large culm. Large culm rice varieties have a higher number of grains per panicle and a longer spike length. Moreover, other morphological and agronomical characteristics for example land the plant height, number of tillers per plant, number of grains per panicle and grain weight have direct effect on grain yield per plant of rice (Selvaraj, C. I., & Nagarajan, P., 2011; Babu, V. R. et al., 2012)

Rice landrace varieties are diverse in the morphological characteristics such as shape, width, grain of length, seed color, leaves color, tongue and stem color (Nantiya, P., & Kansamaporn, T., 2011). According to Habib, S. H. et al. (2005), extent and significance of association of yield with yield components should be considered, while determining the selection criteria of germplasm on the basis of available genetic variations.

Bajracharya, J. et al. (2006) reported variability of agro-morphological traits and SSR markers in rice landrace diversity in Nepal. Rice landrace diversity was low morphological diversity with an average Shannon Weaver diversity index of 0.23 (Bajracharya, J. et al., 2006). In addition, According to While Jamago, J. M., & Cortes, R. V. (2012) seed diversity of traditional rice varieties from Bukidnon, Philippines was assessed using Shannon weaver diversity. Seed weight, grain length and width, grain thickness, caryopsis length and width and lemma showed high diversity (H' 0.76 to 0.88). Hien, N. L. et al., (2007) also studied genetic diversity of morphological responses and the relationships among Asia aromatic rice (*Oryza sativa* L.) cultivars using Shannon-weaver diversity index. Grain size, grain shape, culm strength, plant height and secondary branching contributed the highest mean diversity indices H'=0.91, 0.88, 0.87, 0.82 and 0.83.

Suman, N. (2005) reported the genetic divergence in rice germplasm using 16 quantitative characters. Harvest index contributed maximum to the divergence followed by seed density and total number of tillers per plant. Joshi, S. P. et al. (2000) examined the morphological traits of nineteen varieties of rice and suggested that the variability and heritability coupled with moderate to high genetic advance for most of the morphological traits signified their utility in varietal characterization.

Most of traditional rice varieties have tall structure, low yield, photoperiod-sensitivity and unresponsive to fertilizer (Man, S., 2007). Therefore, tolerant rice cultivars for those stresses have been identified. Information on morphological and agronomical characteristics of these rice cultivars are needed for the future utilization of them in rice improvement and the correlation between yield and other morphological and agronomical characters are important for the production of high yielding rice cultivars programmers (Ranawake, A. et al., 2010; 2011).

CHAPTER III

RESEARCH METHODOLOGY

The present investigation was carried out with an objective to examine morphological and agronomic characteristics of the eight landrace rice varieties. The details of experimental materials and methods used in this study are described below.

The rice plant material

During July 2015, eight landraces, namely Domnerbses (DS) glutinous rice), Neangkert (NK), Laksleuk (LS), Kolphaav (KP), Phrech (PR), Kromounsor (KMS), Chaorng (CH), and Neangkong (Nkong), were collected from Sandan and Prasatsombo of Kampong Thom province, Cambodia (Figure 6) using method generated by Semwal, D. P. et al. (2014). These samples included one glutinous rice variety (Domnerbses) and seven non-glutinous rice varieties (Neangkert, Laksleuk, Kolphaav, Phrech, Kromounsor, Chaorng, and Neangkong. Eight landraces were collected from six farmer's houses in Sandan and Prasatsombo districts, and Names of eight landraces were recognized by farmer. One set of rice landrace samples was used in a field experiment at Sandan district, Kampong Thom province, Cambodia for observation of morphology and agronomic traits and yield component.

Field experiment

The Sandan locates at 12° 46'N latitude and 106° 0'E longitude and Prasatsombo locates at 12° 46'N latitude and 106° 0'E longitude. The climate of study area is subtropical warm and humid in average annual temperature in Kampong Thom is 27.6 °C. The rainfall is 1560 mm averages per year (Ministry of water resources and meteorology Department of meteorology, Cambodia). The collection sites of these rice landrace rice varieties are shown in Figure 6 and the variety name, type and locality are shown in Table 2.

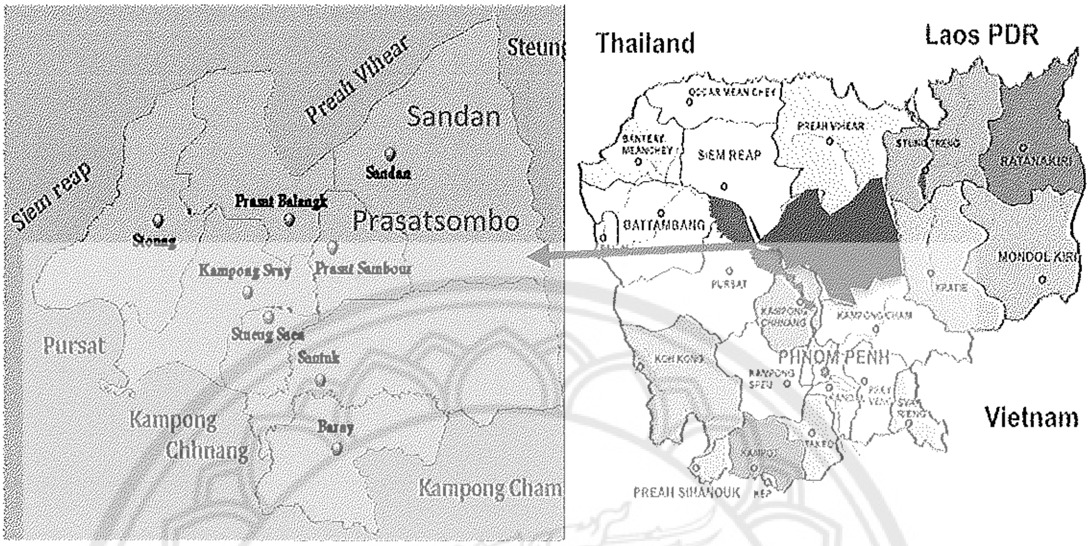

Figure 6 Germplasm sites of eight rice landraces were collected from Sandan and Prasatsombo districts of Kampong Thom Province, Cambodia

Source: Modified from Semwal, D. P. et al., 2014

Table 2 Variability studies in eight rice landraces from Sadan and Prasatsombor district, Kampong Thom Province, Cambodia

No.	Name of the landraces	Collector no.Dist./state	Type of rice	Kernel color	Seed coat color	Presence of awn
1	Neangkert (NK)	Sandan, Kampong Thom	Rain-fed	Straw-red	White	Awnless
2	Laksleuk (LS)	Sandan,Kampong thom	Rain-fed	Straw-red	White	Awnless
3	Kolphaav (KP)	Sandan, Kampong Thom	Rain-fed	Straw	White	Awned
4	Phrech (PR)	Sandan, Kampong Thom	Rain-fed	Straw	Light-brown	Awnless
5	Kromounsor (KMS)	Sandan, Kampong Thom	Rain-fed	Straw - brown	White	Awnless
6	Domnerbses (DS)	Sandan, Kampong Thom	Rain-fed (Waxy)	Straw-brown	White-brown	Awnless
7	Chaorng (CH)	Sombo, Kampong Thom	Rain-fed	Straw-brown	Brown-red	Awnless
8	Neangkong (Nkong)	Sombo,Kampong thom	Rain-fed	Straw-red-	White-red	Awnless

Characterization based on morphology and agronomic traits

One set of rice landrace samples was used for morphological investigations of seeds. Another set of rice landrace samples was grown at the experimental field for yield components and morphological and agronomical investigations of the rice plant.

1. Morphology of seeds

Thirty seeds of rice landraces were randomly selected from each rice landrace variety. Seed length, width, and thickness, seed coat length, seed coat width and seed coat thickness were measured using a digital caliper venire (accuracy ± 0.01 mm) (Armstrong, B. et al. 2005).

The measurement of seed length and width were made for selected grain seed of good shape. Grain types are grouped into round, large and slender types, each corresponding to a-type (japonica), b-type (intermediate type), and c-type (Indica) respectively in Matsuo's tripartite classification of grain types (Matsuo, 1952) (Figure7). Seed and seed coat color were evaluated with standard evaluation system for rice IRRI, (2002).

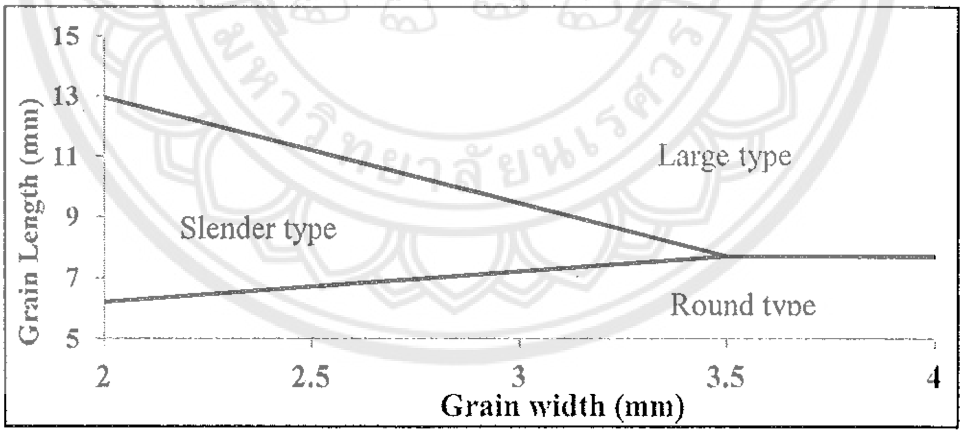

Figure 7 Grouping of grain type of eight traditional Cambodia rice varieties and Classification

Source: Modified from Matsuo, T. 1952; Tadayo et al., 1970; Pradit, A. et al., 2011

2. Observations recorded of morphology and agronomic traits

Eight landrace rice varieties were grown at Sandan district, experimental field in Kampong Thom province, Cambodia under similar management with farmers. The experiment was designed with completely randomized design (CRD), and divided into 8 treatments with 5 replications. The total pots were 40 pots. At 15 day after sowing (DAS), transplant one seedling in each plot for every variety. One plot represents one replication.

Each variety was transplanted fifteen-days-old seedling in a pot. All plants were cultivated in the experimental field during the regular growing season of germplasm and the processes were also noted. Investigations were done for pant height (cm), the number of tillers, the panicle of length (cm), seed per panicle, the tiller per panicle, 1000-grain weight (g), the grain per panicle, grain yield (g), auricle color, leaf color, sheath color, ligule color, leaf margins, seed color and seed coat color, width of leaf (cm), and length of leaf (cm). All measured detailed using random sampling method (Ranawake, A., 2013, Sinha, A. K., & Mishra, P. 2012). Morphology and agronomic traits were recorded at different growth stages for estimation of diversity according to descriptors established by the International Rice Research Institute (IRRI, 1996) as showed in Table 3.

Table 3 Evaluated parameters of data collection in the study

Crop stage	Parameters	Data collection
Seed	Length ,Width and Thickness of seed (mm)	Measured using a digital caliper
	Length ,width and thickness of seed coat (mm)	
	Seed color	Screened by characteristics of kernel color on straw color, straw line, brown on straw, brown, red-purple light, purple on straw, purple and black

Table 3 (cont.)

Crop stage	Parameters	Data collection
	Seed coat color	Taking out of rice husk and screened characteristics of seed coat color and record data on white color, brown light , red, purple line, purple black
Plant	Plant height (cm)	Measured height from the base of the plant to the top of the latest leaf or spikelet
	Tiller number	Counted tillers at the maturity stage
	Leaf color	The evaluate characteristics of leaf color showed such as green, dark green, purple on top of leaf, purple on margin, purple mixed green, and purple.
	Leaf width (cm)	Measure first blade leaf under flag leaf (cm) and randomized 5 simples and statistics analysis
	Length of leaf (cm)	Measure width first blade leaf under flag leaf (cm) in biggest point of leaf and randomized 5 samples and statistics analysis
	Auricle color	Screen by auricle color showed that green light, white and purple
	Sheath color	Screened by the characteristics of sheath color such as green, green purple line , purple light and purple

Table 3 (cont.)

Crop stage	Parameters	Data collection
Plant	Ligule color	Screened by characteristics of ligule color on characterization such as white or non-color , purple line and purple
	Leaf margins	Screened by the margin of length leaf such as white margin, and purple margin
	Leaf angle	Observed on the collar sheath, so between flag leaf with culm of rice such as erect, intermediate, horizontal and descending
	Culm angle	The observed on angle or the character of tiller include Erect <15^{o} Intermediate >15^{o}-30^{o} open >30^{o}-45^{o} Spreading >45^{o}- <90^{o} Procumbent > 90^{o}

Table 3 (cont.)

Crop stage	Parameters	Data collection
Reproductive Harvest Post-harvest	Panicle length (cm.)	Measured from the base of the lowest spikelet of the tip of the latest spikelet on the panicle, excluding awn
	Harvesting time	Counted the day of harvesting time
	Grain per panicle	Counted seed of panicle by plant
	Total yield (g)	Weight of all productivity
	Number of reproductive tillers per plant	The measured by count of tillers with panicles
Reproductive Harvest Post-harvest	1000-grain weight (g)	-1000-grains were counted from five plants of each replicate and weighed. -For 1000-grain weight, random samples of 1,000 well-developed dried to 13% moisture content. One thousand seeds were weighed on a precision balance (ICC, 2004).

Source: Modified from Rabara, R. C. et al., 2014; Jamago, J. M., & Cortes, R. V., 2012; Kumar, S. et al., 2010; IRRI, 2002

Data analysis

Shannon-Weaver diversity index (H) was used to calculate the diversity of the characterized morphology and agronomic traits (qualitative and quantitative traits) following the protocol used by Sotto and Rabara, R. C., (2007). An arbitrary scale was adapted from Jamago, J. M., & Cortes, R. V. (2012) to categorize the computed indices into maximum (H = 1.00), high (H = 0.76–0.99), moderate (H = 0.46–0.75) and low diversity (0.01–0.45). Shanon-weaver diversity index (H') equation is presented as followings:

Shannon Index (H')

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

In this equation, **p** is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), **ln** is the natural log, $\sum$ is the sum of the calculations, and ***s*** is the number of species.

The data in morphological and agronomical traits e.g. seed and seed coat of length, width, thickness and plant height, and the number of tiller were analyzed by two-way analysis of variance (ANOVA) and were subsequently followed by DMRT test using R-program version 3.3.1 (The R Foundation, 2016). Multivariate analysis was performed on qualitative and quantitative data of agro-morphological traits by using principal component analysis (PCA). The PCA was employed to identify the different agro-morphological traits in eight rice landraces that contributed to the most variance in the measured variables. In PCA, the raw data were standardized and the distance matrix using the variance – covariance coefficients was computed (Rabara, R. C. et al., 2014).

CHAPTER IV

RESULTS

The morphology and agronomic traits of eight Cambodia rice landraces were studied during 2016. The results of eight Cambodia landrace rice varieties have been conducted as shown below.

Qualitative traits of morphology and agronomy

1. Seed and seed coat color

The seed and seed coat colors of landrace rice varieties were determined using the IRRI test guideline (Nantiya, P., & Vijittra, A., 2011, Nantiya, P. et al., 2016), the results showed that seed color of rice landraces was classified into six groups. Straw and straw-red group consisted of LS and NK varieties, straw-red of DS, straw, straw-red, straw-brown of Nkong, straw, straw-red, brown-red, straw-brown and spot of CH, straw of KP and PR, and straw –brown of KMS. Those accessions with different seed color are as shown in figure 8.

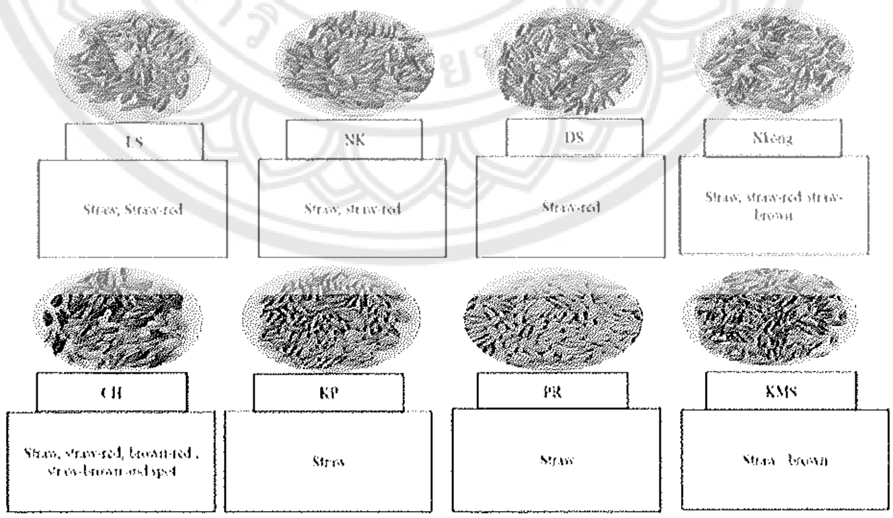

Figure 8 Seed color of eight landrace rice varieties

Note: Prech (PR), Kulphaaw (KP), Neang kong (Nkong), Chorng (CH), Neang kert (NK), Domnerbses (DS), Laksleuk (LS), Kromounsor (KMS)

Seed coat color of eight landrace rice verities revealed that LS, Nkong and KP had white and red colors, CH variety had brown-light and brown colors, KMS and NK had white and white-turbid colors, PR had light – brown colors, and DS had white and turbid colors (Figure 9).

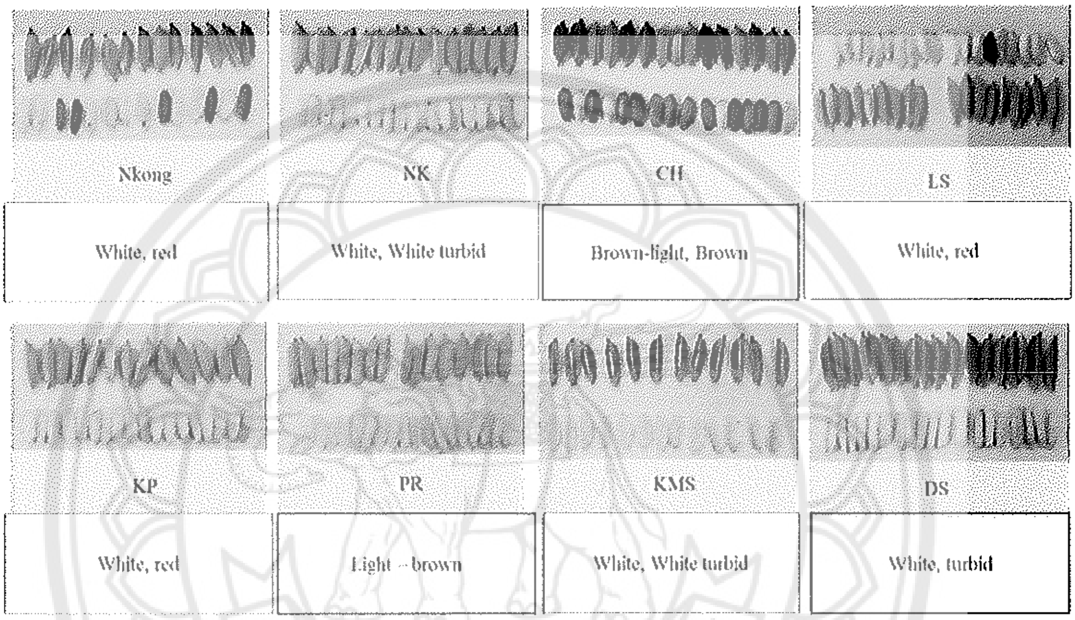

Figure 9 Seed coat color of eight landrace rice varieties

Note: Prech (PR), Kulphaaw (KP), Neang kong(Nkong), Chorng (CH), Neang kert (NK), Domnerbses (DS), Laksleuk (LS), Kromounsor (KMS)

2. Seed type

Results of classification of seed types were showed in figure 9. Classification of seed types consisted of large, round, and slender types. Seeds of eight landrace rice varieties rice varieties had slender type (Figure 10).

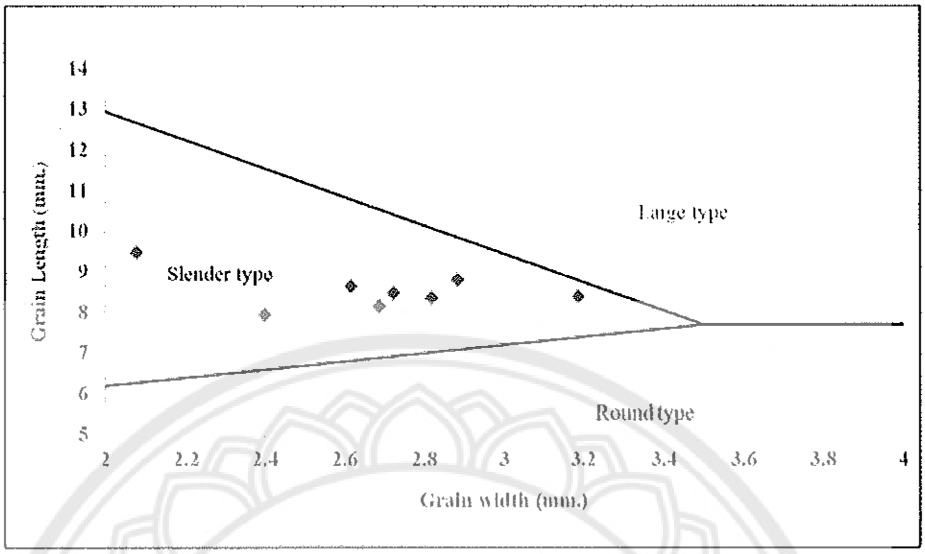

Figure 10 Seed types of eight traditional Cambodia rice varieties

Qualitative traits of agronomic characteristics of eight rice landraces

Agronomic analysis was done using qualitative characteristics collected during the different growth stages of the varieties. These included leaf color, leaf angle, auricle color, ligule color, tiller angle, sheath color, and leaf margin color. The results of agronomic characteristics of eight landrace Cambodia rice varieties from Kampong Thom province were showed in Table 7. The leaf color showed no variability within the varieties evaluated. Eight rice landraces had green leaf blade color (Figure11). The leaf angle of eight-landrace rice varieties showed that LS, Nkong, KMS, and DS varieties were intermediate traits, while CH, KP, NK, and PR varieties were erect trait (Figure11). The auricle colors ranged from white-purple (LS variety), purple green (KP and Nkong varieties), purple (CH, KMS, and DS varieties) and white (NK and PR variety). The result of ligule color showed that LS, Nkong, and PR varieties were green color, while KMS and NK varieties were green purple colors. In addition, purple color was found in DS variety, purple green color in KP variety, and purple green white colors in CH variety. Moreover, the sheath colors of eight landrace rice varieties were found in five colors including green purple color in LS, CH, and KP varieties, white-green purple color in KMS variety, purple color in DS variety and the green color in Nkong and PR varieties. For the tiller angle, the intermediate trait was observed for LS, Nkong, KP, KMS, NK, PR, DS varieties, and while open trait was found in CH variety (Figure12). In terms of leaf margin color, seven accessions viz. LS, Nkong, CH, KMS, NK, PR and DS landraces were white and only one accessions viz. KP landraces was purple white (Figure12).

Table 4 The qualitative traits of agronomic characteristics of eight rice landraces in Kampong Thom province

Varieties	Leaf		Auricle color	Ligule color	Tiller angle	Sheath color	Leaf margin color
	Color	Leaf angle					
LS	Green	Intermediate	White, Purple	Green purple	Intermediate	Green	White
Nkong	Green	Intermediate	Purple green	Green	Intermediate	Green	White
CH	Green	Erect	Purple	Purple green	Open	Purple green white	White
KP	Green	Erect	White purple	Purple green	Intermediate	Purple green	Purple white
KMS	Green	Intermediate	Purple	White-green purple	Intermediate	Green purple	White
NK	Green	Erect	White	Green white-green	Intermediate	Green purple	White
PR	Green	Erect	White	Green	Intermediate	Green	White
DS	Green	Intermediate	Purple	purple	Intermediate	Purple	White

Note: Prech (PR), Kulphaaw (KP), Neang Kong (Nkong), Chorng (CH), Neang kert (NK), Domnerbses (DS), Laksleuk (LS), Kromounsor (KMS)

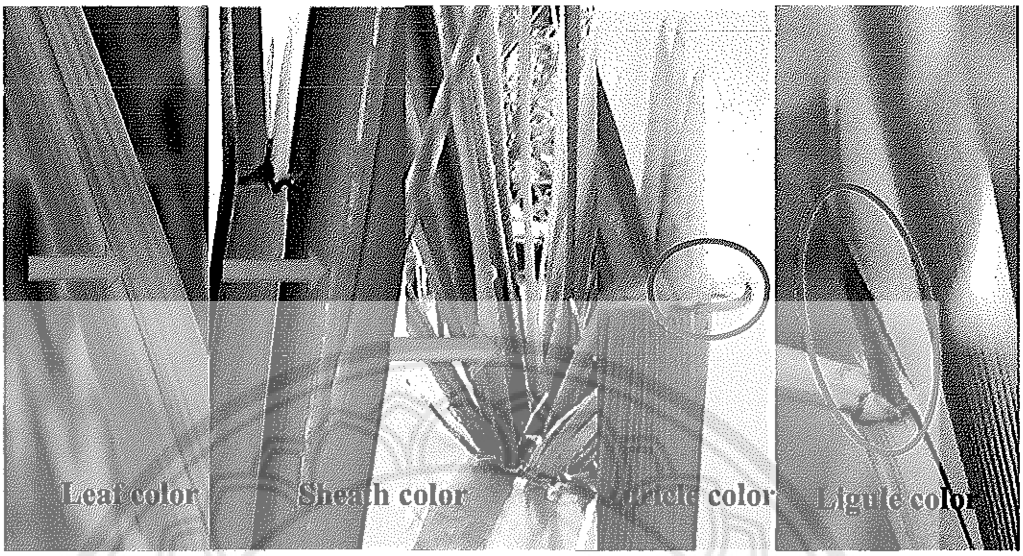

Figure 11 The agronomic of eight landrace rice varieties in Kampong Thom Province

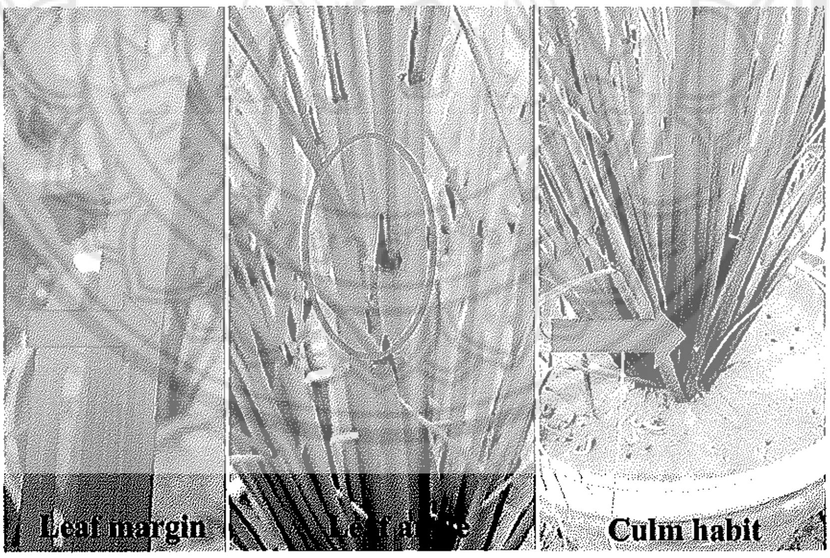

Figure 12 Some qualitative traits of agronomic characteristics of eight landrace rice varieties in Kampong Thom province

Note: 1. Erect (<15°), 3. Intermediate (~20°), 5. Open (~40°), 7. Spreading (>60-80°) 9. Procumbent:

Source: IRRI

Quantitative traits of morphology and agronomy

1. Quantitative traits of morphological characteristics of seed and seed coat

Some morphological characteristics of eight landrace rice varieties from two districts of Kampong Thom province, Cambodia are shown in Table 5. The results indicated that all tested seed-morphological characteristics were highly significant differences at *p* value 0.001 (table 5). Lengths of seeds ranged from 7.9 to 9.5 mm. The longest seed was obtained in Domnerbses (DS) variety. Average value was found to be 8.5 mm. The seeds of Chaorng (CH) variety were recorded with a maximum width of 3.2 mm, and a maximum thickness of 2.3 mm. Ratios of seed length to seed width varied from 2.6 to 4.6. Minimum seed ratio was recorded for Chaorng (CH) variety with an average value of 3.3.

2. Quantitative traits of agronomic characteristics and yield component

Investigations were done for plant height, tiller number, length of panicle, seed per panicle, harvesting time, tiller per panicle, 1,000-grain weight, total weight, width of leaf, and length of leaf. Measurements were done at 75 days after transplanting. The results are given in Table 6. The results indicated that plant height, tiller number, length of panicle, seed per panicle, harvesting time, tiller per panicle, 1000-grain weight, total weight, and width of leaf were highly significant differences at *p* value 0.001. No significant differences were observed in length of leaf ($p < 0.05$).

Plant height ranged from 145.2 to 205.4 cm. Maximum plant height was recorded for Chaorng (CH) variety. Tiller numbers were observed between 20.6 to 31.0. The lowest value for tiller numbers was recorded for Kulphaaw (KP) variety. Length of panicle ranged from 22.6 to 30.2. The lowest value for panicle was recorded for Kromounsor (KMS) variety. Seed per panicle ranged from 150.8 to 245.2. Maximum seed per panicle was recorded for Neang kert (NK) variety. Harvesting time ranged from 104 to 129 days. Maximum harvesting time was recorded for Chaorng (CH) variety. Minimum harvesting time was recorded for Neang kert (NK) and Kulphaaw (KP) varieties. Tiller per panicle ranged from 0.4 to 0.7 %. Maximum tiller per panicle was recorded for Laksleuk (LS) variety, however, it was not significant difference from Chaorng (CH), Kromounsor (KMS), Neang kert (NK), and Neang Kong (Nkong) varieties. 1,000-grain weight ranged from 18.8 to 25.5 g. The highest value for 1,000-grain weight was recorded for Chaorng (CH) variety. For total weight, it ranged from 74 to 155.5 g. Maximum total grain weight was recorded for Neang kert (NK) variety followed by Kromounsor (KMS) variety, and Laksleuk (LS) variety, respectively. Width of leaf ranged from 0.9 to 1.2 cm, while length of leaf was observed between 62.8 to 69.4 cm.

Table 5 The quantitative traits of morphological characteristics of seed and seed coat in eight varieties of rice from Kampong Thom province, Cambodia

Varieties	Seed			Seed coat (mm)			Seed[2)] length ratio
	Length (mm)	Width (mm)	Thickness (mm)	Length (mm)	Width (mm)	Thickness (mm)	
LS	$8.6^{1)\ bc}$	2.6^{e}	2.0^{b}	6.3^{b}	2.4^{c}	1.8^{bc}	3.3^{b}
KP	8.4^{cd}	2.7^{cd}	2.1^{b}	6.1^{bc}	2.4^{bc}	1.9^{b}	3.1^{c}
CH	8.4^{d}	3.2^{a}	2.3^{a}	6.1^{bc}	2.9^{a}	2.1^{a}	2.6^{d}
KMS	8.8^{b}	2.9^{b}	2.0^{b}	6.3^{b}	2.5^{b}	1.9^{bc}	3.1^{c}
PR	7.9^{e}	2.4^{f}	1.8^{d}	5.9^{c}	2.1^{d}	1.7^{cd}	3.1^{c}
DS	9.5^{a}	2.1^{g}	1.8^{d}	6.9^{a}	1.9^{e}	1.6^{d}	3.0^{c}
Nkong	8.0^{e}	2.7^{de}	2.0^{bc}	6.0^{c}	2.5^{bc}	1.8^{bc}	3.3^{b}
NK	8.3^{d}	2.8^{bc}	1.9^{c}	6.1^{bc}	2.4^{b}	1.8^{c}	4.6^{a}
Mean	8.5	2.7	2	6.2	2.4	1.8	3.3
CV (%)	2	3	2.3	3.2	3.7	3.4	7.5
F-test	**	**	**	**	**	**	**

Note: Prech (PR), Kulphaaw(KP), Neangkong (Nkong), Chorng (CH), Neangkert (NK), Domnerbses (DS), Laksleuk (LS), Kromounsor (KMS).

[1]The values are expressed as mean.

Different lower case letters (in same column) correspond to significant differences at $P < 0.01$.,** significant difference at $P< 0.01$.

[2]Seed length ratio is Seed length/seed width

Table 6 Agronomic characteristics and yield components of eight landrace rice varieties

Varieties	Plant height (cm)	Tiller number	Length of panicle (cm)	Seed per panicle	Harvesting time (days)	Tiller per panicle (%)	1,000-grain weight (g)	Total weight (g)	Width of leaf (cm)	Length of leaf (cm)
LS	$159.2^{1/cd}$	29.6^{a}	26.8^{b}	199^{b}	128^{b}	0.7^{a}	21.9^{d}	128.6^{abc}	0.9^{d}	62.8
KP	151.6^{cd}	20.6^{b}	28.2^{ab}	160^{bc}	123^{c}	0.4^{c}	22.4^{c}	74.0^{d}	1.1^{ab}	67.4
CH	205.4^{a}	28.2^{a}	29.8^{ab}	150.8^{c}	129^{a}	0.6^{ab}	25.5^{a}	108.7^{bc}	1.2^{a}	63.4
KMS	160.8^{c}	30.6^{a}	22.6^{c}	186^{bc}	128^{b}	0.6^{ab}	23.5^{b}	134.8^{ab}	0.9^{d}	64.4
PR	155^{cd}	25.4^{ab}	29.6^{ab}	152.4^{c}	104^{d}	0.5^{bc}	18.8^{g}	72.9^{d}	1.2^{a}	69.4
DS	145.2^{d}	29.0^{a}	27.6^{ab}	169.2^{bc}	104^{d}	0.5^{bc}	19.7^{f}	97.1^{cd}	1.1^{abc}	66.2
Nkong	176.2^{b}	31.0^{a}	28.4^{ab}	168.4^{bc}	128^{b}	0.6^{abc}	20.9^{e}	109.0^{bc}	0.9^{cd}	68.0
NK	163.0^{bc}	28.2^{a}	30.2^{a}	245.2^{a}	123^{c}	0.6^{abc}	22.5^{c}	155.5^{a}	1.0^{acd}	67.2
Mean	164.6	27.8	27.9	178.9	120.9	0.56	21.9	110.1	1.0	66.1
CV (%)	6.6	14.9	7.5	16.2	9.1	0.6	1.5	22.3	9.2	7.7
F-test	**	**	**	**	**	**	**	**	**	ns

Note: Prech (PR), Kulphaaw (KP), Neangkong (Nkong), Chorng (CH), Neangkert (NK), Domnerbses (DS), Laksleuk (LS), Kromounsor (KMS). [1)] The values are expressed as mean. Different lower case letters (in same column) correspond to significant differences at $P < 0.01$., ** significant difference at $p < 0.01$. ns = the difference is not significant ($p > 0.05$)

Diversity in qualitative and quantitative trails

In this study, the qualitative traits (seed color, seed coat color, and leaf color), and the quantitative traits (seed length (mm), seed length ratio (L/W), seed coat length (mm), and seed coat ratio (L/W) were used to assess the diversity. In order to evaluate the diversity, Shannon-Weaver Diversity Index calculation was chosen. The calculation was based on the individual raw data of eight landrace rice varieties. In Table 7, the results present the Shannon-Weaver diversity indices (H′) of the nine qualitative and quantitative agro-morphological traits. Overall, leaf color of eight rice landraces showed extremely high levels of diversity for the qualitative and quantitative traits ranging from 1.6 to 2.0. The highest leaf color variability was found in KP variety. Low to moderate diversity indices were observed for seed length ratio and seed coat length with indices ranging between 0.2 to 0.7. The highest diversity indices of seed (H′= 1.4) and seed coat (H′= 0.7) colors were found in CH variety. For seed length, PR variety was the highest diversity index (H′= 0.45). For seed length ration, moderately diverse traits were observed for KP, LS, NK, Nkong and PR varieties with indices ranging between 0.5 to 0.7, while non-variability was found in CH variety. In term of seed coat length ratio, CH variety was the highest diversity (H′= 0.7). KP and PR varieties showed non-variability of diversity for leaf blade width and leaf blade length.

Table 7 Diversity index (H') of qualitative traits of eight landrace rice verities

Varieties	Diversity index of qualitative traits (H')								
	Seed						Leaf		
	Seed color	Seed length	Seed length ratio	Seed coat color	Seed coat length	Seed coat length ratio	Leaf color	Leaf blade width	Leaf blade length
LS	0.2	0.0	0.6	0.2	0.7	0.3	1.8	0.7	0.0
Nkong	1.3	0.2	0.6	0.5	0.5	0.2	1.7	0.7	0.0
CH	1.4	0.2	0.0	0.7	0.4	0.7	1.6	0.0	0.5
KP	0.0	0.0	0.6	0.2	0.6	0.3	2.0	0.0	0.0
KMS	0.0	0.0	0.2	0.0	0.2	0.0	1.8	0.7	0.7
NK	0.3	0.2	0.5	0.0	0.5	0.0	1.9	0.5	0.5
PR	0.0	0.5	0.7	0.0	0.5	0.2	1.6	0.0	0.0
DS	0.0	0.0	0.2	0.0	0.5	0.0	1.6	0.0	0.5

Note: high variability = 0.76 – 0.99 moderate variability =0.46 – 0.75, low / poor variability = 0.01–0.4, non-variability =0.00

Source: Jamago, J. M., & Cortes, R. V., 2012

Pearson' s Correlation analysis of coefficient among 16 quantitative traits of eight landraces of rice varieties

Using Pearson's correlation, an analysis was done to assess the relationship among the 16 quantitative traits of eight rice landraces varieties. The relationship among characteristics is represented in Table 8. An analysis showed that plant height was a positive and strongly significant correlation with seed width (r= 0.69), seed thickness (r=0.70), seed coat width (r=0.73), seed coat thickness (r=0.71), while the correlation between the plant height and the harvesting time (r=0.50), 1000-grain weight (r=0.58) was moderate. Tiller number showed a moderate positive and strongly significant correlation with tiller per panicle (r=0.66), and total weight (r=0.66). Moreover, weak negative correlations were found between tiller number with length of panicle (r=-0.34), and width of leaf (r=-0.39). Seed length had a strong positive and strongly significant correlation with seed coat length (r= 0.87), while weak negative correlations were found between seed length and seed width length (r=-0.31), and seed coat width (r=-0.33). Seed width was found to have strong positive significant correlations with seed thickness (r= 0.90), seed coat width (r=0.96), seed coat thickness (r=0.84), harvesting time (r=0.80) and 1000-grain weight with (r=0.88). Seed thickness character showed strong positive significant correlations with seed coat width, seed coat thickness, harvesting time, 1000-grain weight with r=0.92, r=0.93, r=0.78 and r=0.88, respectively. Seed coat width was weak negative significant correlations with seed coat length (r= -0.35). Moreover, the seed coat width showed strong positive correlations with seed coat thickness (r=0.89), harvesting time (r=0.82), and 1000-grain weight (r=0.86). The seed coat thickness showed positive significant correlations with harvesting time (r=0.69), 1000-grain weight (r=0.83). A moderate positive correlation was observed between length of panicle and width of leaf. Total weight of eight landrace rice varieties showed strong positive significant correlation with seed per panicle (r=0.80). However, total weight of eight landrace rice varieties showed positive correlation significant with harvesting time, tiller per panicle and 1000-grian weight with r = 0.42, r = 0.47 and r=0.37, respectively. Therefore, the positive correlation showed that 1000-grain weight (g) tended to increase with the increasing plant height, seed width, seed thickness, seed coat width, seed coat thickness, harvesting time and tiller par panicle.

Principal component analyses of eight landrace rice varieties

Table 9 presents the principal component and percentage contribution of each component to the total variation in 16 characteristics of eight landrace rice varieties. The two dimensions accounted for 70.6% of the whole variation with PC1 and PC2 contributing 45.2% and 25.4% of the total variance, respectively (Figure 13). Highly and positively correlated variables with PC1 were plant height, seed width, seed thickness, seed coat width, seed coat thickness, harvesting time, tiller per panicle, 1000-grain weight and total weight with loading of 0.81, 0.94, 0.93, 0.96, 0.90, 0.90, 0.67, 0.92 and 0.51, respectively (Table 9; Figure 13). 1000-grain weight, seed width, seed coat width, seed coat thickness, plant height and seed thickness were strongly positively correlated to PC1 as well as Nkong and CH varieties. The principal component of PC2 was associated with tiller number (0.68), seed length (0.68), seed coat length (0.66) seed per panicle (0.56), and total weight (0.67). Seed per panicle, tiller number, total weight, tiller per panicle and harvesting time had strongly positive correlation to PC1 as well as LS, KMS and NK varieties. Plant height, seed width, seed thickness, seed coat width, seed coat thickness, length of panicle, width of leaf, and length of leaf showed negative correlation with PC2 as well as Nkong, PR, KP, and CH varieties.

All these vegetative and reproductive characters gave contribution to the components. In most of the cases, PC analyses showed similar correlation among traits and varieties as correlation test.

Table 8 Correlation coefficient among 16 quantitative trait of eight landraces rice varieties

Parameter	PH	TN	SL	SW	ST	SCL	SCW	SCT	LP	SP	HT	TP	1000 GW	TW	WL
PH	-														
TN	0.21	-													
SL	-0.28	0.18	-												
SW	0.69**	0.03	-0.31*	-											
ST	0.70**	0.04	-0.15	0.90**	-										
SCL	-0.37*	0.16	0.87**	-0.37*	-0.19	-									
SCW	0.73**	0.02	-0.33*	0.96**	0.92**	-0.35*	-								
SCT	0.71**	0.05	-0.15	0.84**	0.93**	-0.14	0.89**	-							
LP	0.20	-0.34*	-0.35*	0.01	-0.03	-0.28	0.04	0.00	-						
SP	-0.12	0.20	0.12	0.07	-0.11	0.08	0.00	-0.09	-0.10	-					
HT	0.50**	0.17	-0.19	0.80**	0.78**	-0.24	0.82**	0.69**	-0.19	0.19	-				
TP	0.36*	0.66**	0.07	0.30	0.30	0.02	0.32*	0.21	-0.13	0.07	0.38*	-			
1000GW	0.58**	0.08	0.03	0.88**	0.88**	-0.06	0.86**	0.83**	-0.11	0.1	0.78**	0.32*	-		
TW	0.16	0.66**	0.18	0.29	0.16	0.11	0.23	0.18	-0.26	0.80**	0.42**	0.47**	0.37*	-	
WL	0.16	-0.39*	-0.13	-0.05	-0.03	-0.09	-0.03	0.08	0.47**	-0.29	-0.38*	-0.43**	-0.07	-0.41**	-
LL	-0.07	-0.14	-0.23	-0.17	-0.21	-0.14	-0.16	-0.11	0.14	0.04	-0.24	-0.17	-0.25	-0.08	0.04

Note: (**) and (*) indicate $P<0.01$ and $P<0.05$ level of significance respectively. Plant height (PH), Tiller number (TN), Seed length (SL), Seed width (SW), Seed thickness(ST), Seed coat length (SCL), Seed coat width(SCW), Seed coat thickness (SCT), Length of panicle(LP), Seed per panicle (SP), Harvesting time (HT), Tiller per panicle (TP),1000- grain weight(1000GW), Total weight (TW), Width of leaf (WL), Length of leaf (LL). weak ($r < 0.35$), moderate ($r = 0.36–0.67$) and strong ($r = 0.68–1.00$) correlations

Table 9 Results of the Principal Component Analysis applied to variables (16 quantitative traits) and Pearson's Correlation between the Principal Component scores and 16 quantitative trait of eight landraces rice varieties

Variables	PC1	PC2
Plant height (PH)	0.81	-0.33
Tiller number (TN)	0.28	0.68
Seed length (SL)	-0.19	0.68
Seed width (SW)	0.94	-0.25
Seed thickness (ST)	0.93	-0.26
Seed coat length (SCL)	-0.28	0.66
Seed coat width (SCW)	0.96	-0.27
Seed coat thickness (SCT)	0.90	-0.33
Length of panicle (LP)	-0.10	-0.65
Seed per panicle (SP)	0.13	0.56
Harvesting time (HT)	0.90	0.13
Tiller per panicle (TP)	0.67	0.49
1000- grain weight (1000GW)	0.92	-0.01
Total weight (TW)	0.51	0.67
Width of leaf (WL)	-0.23	-0.78
Length of leaf (LL)	-0.66	-0.50
Proportion of variance %	45.17	25.44
Cumulative variance %	45.17	70.61

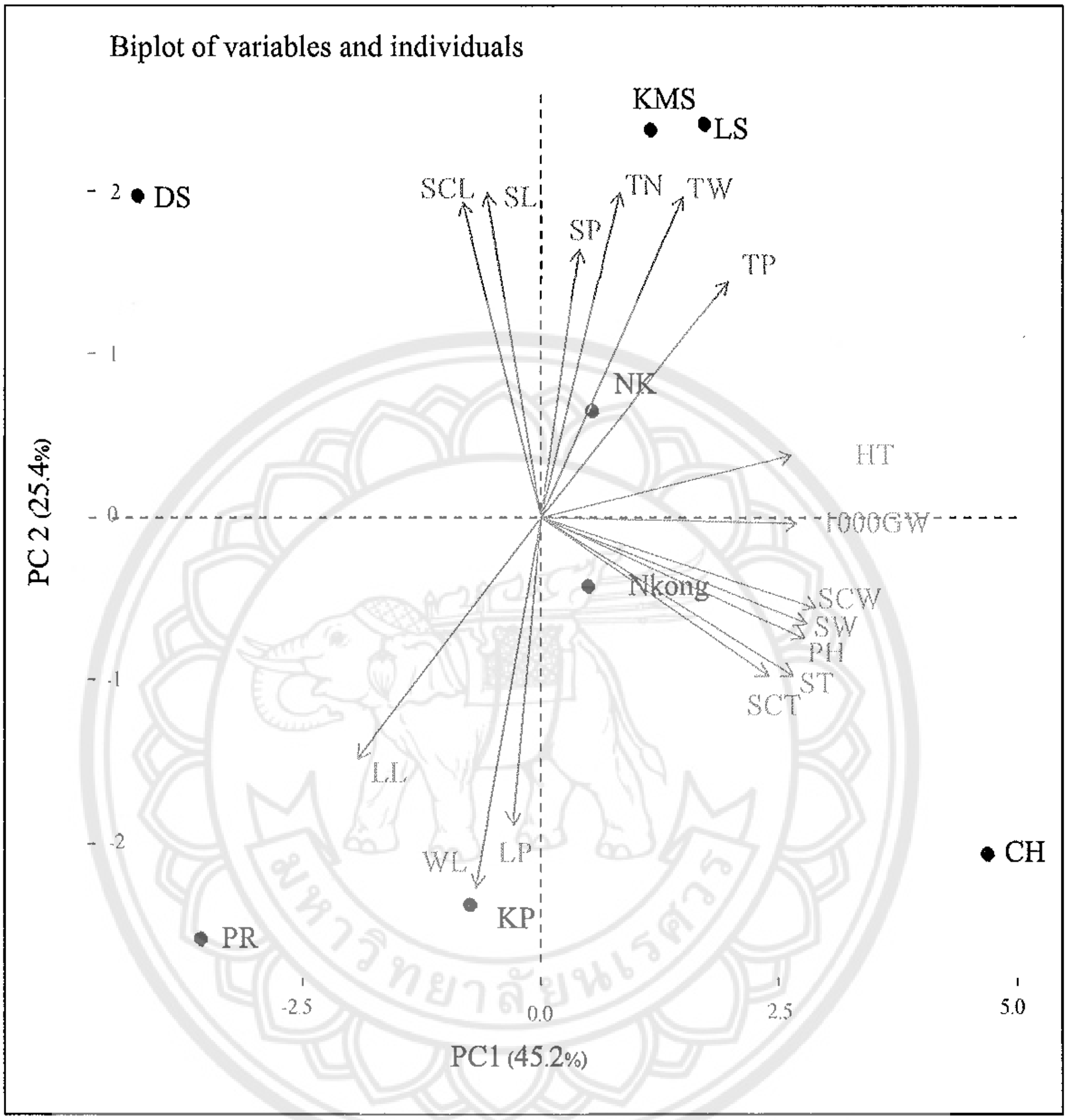

Figure 13 PC analysis of 16 vegetative and reproductive characters among 8 cultivars

Note: Prech (PR), Kulphaaw (KP), Neang kong(Nkong), Chorng (CH), Neang kert (NK), Domnerbses(DS), Laksleuk (LS), Kromounsor (KMS), Plant height (PH), Tiller number (TN), Seed length(SL), Seed width (SW), Seed thickness(ST), Seed coat length (SCL), Seed coat width(SCW), Seed coat thickness (SCT), Length of panicle(LP), Seed per panicle (SP), Harvesting time (HT), Tiller per panicle (TP), 1000- grain weight(1000GW), Total weight (TW), Width of leaf (WL), Length of leaf (LL)

CHAPTER V

CONCLUSION

Discussion

The characteristics of eight traditional Cambodia rice varieties from Kampong Thom province, were shown in (Table 3). Among the test of eight traditional rice cultivars had recorded plant heights between 103 – 125cm while the highest of rice cultivars in Chorng (CH) varieties had value of 125.4 cm. According to the standard evaluation system for rice the definition (IRRI, 1988; IRRI, 1996). The shortest of eight traditional rice varieties, was Damnerbses (DS), had a recorded value of 103 cm for plant height. The height of eight traditional rice varieties between 103 -125 cm is in the intermediate standard of biodiversity, (IRRI, & WARDA, 2007). The rice tillering is an importance for yield component. Tiller number of eight traditional rice varieties had evaluated average between 43- 57 tillers numbers so this is early tiller number on vegetative trait, the highest of rice landraces in Damnerbses (DS) varieties had recorded value of 57 tiller number and the less then of tiller number was Lakslerk (LS) 43 tiller number and the standard of tiller number is >20 culms showed the high of tiller allowing with biodiversity (IRRI, & WARDA, 2007; Martinez- Eixarch et al. , 2015) . For characteristics of flag leaf and their relationships with yield and yield traits (Yue et al., 2006) it is important to genetically analyze the morphological and the physiological characteristics of functional leaves, especially flag leaf, in rice improvement. The highest value for the leaf of eight traditional rice varieties in leaf width was recorded for Chorng (CH) and Prech (PR). Krormounsor (KMS) had the lowest value of leaf width 0.92 cm and for the leaf length showed an average leaf length of the Prech (PR) variety about 69.4 cm. Lakslerk (LS) variety lowest of leaf length at 62.8 cm. The size of leaf areas is a source for photosynthesis to develop stem followers and fruit or yield (Teng et al., 2004; Yue et al., 2006). And for the leaf color of eight rice landraces found in green colors according with Teng et al. (2004), high chlorophyll content and delaying senescence of the leaves have been considered to be a favorable characteristic in crop production. The leaf angle of eight traditional rice varieties was found in LS, Nkong, KMS, DS which these varieties were intermediate trait. CH, KP, NK, and PR varieties were found in erect trait. The auricle color of eight traditional rice varieties found in white purple color for LS and KP. Nkong was purple green color and CH, KMS, DS were purple color and NK and PR were white color. Ligule color of eight traditional rice varieties were found white color for LS, Nkong, CH, KMS, NK, PR and DS. Only one variety was purple white color in KP (Supamongkol, 2006).

The results indicated of seed of eight traditional Cambodia rice varieties was classified as seed coat length in seven varieties such as LS, Nkong, CH, KP, KMS, NK, and PR was medium characters grain and DS variety was long grain. Also the body of seed coat of seven varieties such as LS, Nkong, CH, KP, KMS, NK, and PR were medium characters' grain, and DS variety was slender characters grain. The ratio of grain length or kernel of eight traditional Cambodia rice varieties considering of seed coat length and body seed coat of seven varieties both size were short bold and one variety had long slender kernel. The ratio of grain length of eight traditional rice verities found extra-long grains characters. The ratio of body grain was LS (medium grain) and Nkong, NK, KP, DS had slender grains and CH, KMS, PR found in bold grains. So the eight traditional rice varieties had difference seed ratio on the medium, long, extra-long, slender, and bold. The diversity of grain and seed coat colors of eight traditional rice varieties was also evident with all state being represented in the rice varieties evaluated. White seed coat color and grain color of CH was highest diversity and DS varieties was non-diversity (IRRI, 1988; IRRI, 1996; Bioversity, IRRI, & WARDA, 2007; Nantiya, P., & Kansamaporn, T., 2011)

Seed of eight traditional Cambodia rice varieties was classified as seed coat length in seven varieties such as LS, Nkong, CH, KP, KMS, NK, and PR was medium characters grain and one DS variety was long grain. And also the body of seed coat of seven varieties such as LS, Nkong, CH, KP, KMS, NK, and PR were medium characters grain and DS variety was slender characters grain followed by Eram et al., (2014). The ratio of grain length or kernel of eight traditional Cambodia rice varieties considering of seed coat length and body seed coat of seven varieties both size were short bold and one variety had long slender kernel. The ratio of grain length of eight traditional rice verities found extra-long grains characters. The ratio of body grain was LS (medium grain) and Nkong, NK, KP, DS had slender grains. CH, KMS, PR found in bold grains. The diversity of grain and seed coat colors of eight traditional rice varieties the was also evident with all state being represented in the rice varieties evaluated. White seed coat color and grain color of CH was highest diversity and DS varieties was non-diversity (Supamongkol, 2006). Grain yield is one of the important aims in conventional crop breeding. However, grain yield, as well as yield components, is an extremely complex trait, and genetic control of grain yield is realized through the control of a series of complex biochemical and physiological processes (Ashraf et al, 1994). So Eight varieties of rice landraces were collected from Kampong Thom province. Means for grain length, width and thickness from 8 varieties of rice landraces were 8.52, 2.67, and 1.99 mm, respectively. Based on the recorded data for the length of traditional rice grain from India by Semwal, D. P. (2014), the similar result has also been found in this study. In this study, the eight rice landraces from Kampong Thom province, Cambodia were grouped into two categories as medium and long based on the hulled grain length (IRRI, 2013). The longest grain was observed in the variety Domnerbses (DS) about 9.49 mm, but it had low value of width and thickness. The result indicated that the longer grain tend to be narrow, which corresponded with the previously finding of Patra, (2000; Deb, & Battacharya, 2005). Moreover, out of 8 local landraces from Kampong Thom province highest 1,000-grain weight was found in cultivar, Chaorng (CH) (23.59 g) collected from Sombo district, and lowest grain weight was found in Domnerbses (DS) (19.12 g). 1,000-grain weight of rice landraces from Kampong Thom province was similar result with 1,000-grain weight of traditional rice from Bangladesh (A.K.M. Golam Sarwar et al., 1998). Eight traditional Cambodia rice varieties from Kampong Thom province, were collected by interviews of farmers. The two qualitative seed traits such as grain color and seed coat color (table 4). And the quantitative seed traits in (table 5) such as grain length (mm), grain length ratio (L/W), seed coat length (mm), and seed coat ratio (L/W) However , Shannon-Weaver Diversity Index (SWDI) calculate that were based on the individual raw data of eight varieties showed Jamago, J. M., & Cortes, R. V. , 2012; Rabara, R. C. et al. (2014).Grain color: the first of seven qualitative trait of eight traditional rice varieties was highly diversity in straw color of grain with H' = 1.67. However, the divers trait of hull color (H'=1.67) of eight rice landraces from Kampong Thom province was higher than hull color (H'=0.84) from Thale Noi Basin of Phatthalung province, Thailand (Nantiya, P., &

Vijittra, A., 2011), moderate diversity was H'= 0.50 in Straw brown spot, low diversity with Brown-red, Straw and red, Light green, Straw and brown and non-diversity at H"= 0.45, H'=0.41, H'=0.38 and H'=30, respectively and H'=00. Similar result was obtained by Nantiya, P., & Vijittra, A. (2011). And all most of grain color is straw color followed by Jamago, J. M., & Cortes, R. V., 2012). Seed coat color: When peel of lemma and palea found five qualitative traits in seed coat color. The white color was highest diversity H'=1.61, High Variability H'=0.92 in red color, Moderate diversity H' = 60 in brown light color and brown white light found non-diversity H'=0. For qualitative trait seed of grain length classified to four characters (Table 5). Eight traditional of Cambodia rice was extra-long and long grain length had high diversity H'= 1.94 and H'=1.09, respectively and medium and short grain had non-diversity H'=00. The character of grain length ratio of eight traditional rice varieties was high diversity seed ratio in bold and medium types at H'=1.88 and H'=1.61, respectively and slender type is non-diversity. And contrary with seed coat length found high diversity in long, medium and short H'=1.49, H'=1.94 and H'=1.59 and seed coat length non-diversity in extra-long grain. (Table 5). All so the character of seed coat length ratio of eight traditional rice varieties was high diversity seed ratio in medium and slender at H'=1.93 and H'=0.95, moderate diversity in bold seed at 0.51 and round short types is non-diversity. (Nantiya, P. et al., 2016, Jamago, J. M., & Cortes, R. V., 2012, Rabara, R. C. et al., 2014).

Using Pearson's correlation is measure of strength of linear relationship in between among 14 parameters of eight traditional rice varieties. The relationship and correlation in between character are represented in Table 5. The result of correlation analysis showed in table 5 reveals that there was positive and highly significant correlation between 1000-grain weights with grain width (r= 0.88), grain thickness (r=0.88), seed coat width (r=0.86), seed coat thickness (r=0.83), length of panicle (r=0.38), harvesting time (r=0.78) and tiller per plant (r=0.51) and negative correlation with tiller number (r= -0.34). (Taylor, 1990). The characters had positive relationship with 1000-grain weight were plant height (r=0.31), grain length (r=0.03). Among these characters only seed coat length (r=-0.06), seed per panicle (r=-0.07) was non-significant correlation. Plant height had positive significant correlations with all characters except harvesting time, tiller per plant, 1000-grain weight and weight. In the same time, grain length and seed coat length had negative significant correlation. And among these character only tiller number showed negative non-significant correlation with plant height. Negative and significant correlation were shown for tiller number with grain width, grain thickness, seed coat width , harvesting time , tiller per panicle and 1000-grain weight (r= - 0.36 ,r -0.34 , r = - 0.40 , r= - 0.32 , r= - 0.23 , and r= - 0.34 , respectively). However, tiller number positive but non-significant correlation with grain length, seed coat length (r = 0.15, r = 0.19), respectively. The negative correlation show that 1000-grain weight (g) and weight tended to increase as also between weights with seed per panicle to positive significant correlation. While the negative correlation between 1000- grain weight with tiller number and to tended decrease. These trait that had moderate to high correlations could be used as a basic for the utilization for germplasm (Chen, Y., & Lübberstedt, T., 2010).

Conclusion

In conclusion, the present genetic study reveals that:

Traditional rice varieties with eight samples have the variability in morphological characteristics, the height of rice was developing from one growth period to other period quickly. Different varieties with different tiller numbers were growing fast until 75 days and stopped then after for flowering. Considerable genetic diversity exists in the eight traditional rice populations of two locations in Cambodia, although the diversity level varied among populations and between populations, which is most likely due to different conditions, under which the populations are grown and conserved. Morphological characters of eight traditional rice was cultivated rice were found, such as seed type, leaf sheath, grain color, seed coat color, auricle color, leaf color, leaf margin, ligule color, leaf length, leaf width and seed ratio were controlled by few major germplasm with actions and plants segregated within the range of cultivated. The region has traditional varieties with diversified uses, including few slender grained of type seed. The adequate variation in seed trait may be useful for future rice breeding programs that objectives improve varieties for upland rice ecosystem and the information of these morphological and agronomic characteristics can be used for further germplasm selection and improvement of rice variety.

Suggestions

It is highly suggested that further collection of traditional Cambodia rice varieties from other upland agro- ecosystems in Cambodia should be done to ascertain the available genetic resources. There are increasing threats to these germplasm such as natural calamities, land use conversion, and climate change, henceforth, their collection could be of top priority.

Further, it is recommended that morpho-agronomic assessment be done on the collected genotypes in one location, and to determine if there are duplicates in the collected materials and those that will be collected in the future. These are simple ways to contribute to the goal of food (rice) security in our country

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