Songklanakarin J. Sci. Technol.
38 (5), 495-500, Sep. - Oct. 2016

http://www.sjst.psu.ac.th

Original Article

New data on the domestication of the two subspecies *indica* and *japonica* of the Asian cultivated rice (*Oryza sativa*) during the Dvaravati Period in Thailand and Lao PDR

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Received: 21 October 2015; Accepted: 21 January 2016

Abstract

Rice hull imprints on bricks of the Dvaravati Period from four historical sites in Thailand and Lao PDR were measured and classified. In addition, a collection of traditional rice landraces from northern, central, northeastern regions of Thailand, Lao PDR, Vietnam and Cambodia were used to examine rice grain shapes and to test phenol reaction response of rice hulls. The results show that rice hulls in bricks from the Dvaravati Period may be classified into three types of cultivated rice grain shapes, i.e., round, large, and slender types. With the combined methods in archaeology, physiology and ecotype, the present domesticated rice landraces have been combined and by implication, can be possibly traced to types of rice in the Dvaravati Period. It is now suggested that the subspecies *indica* and *japonica* of Asian cultivated rice were domesticated at that time.

Keywords: rice domestication, historical sites, Dvaravati Period

1. Introduction

The Asian cultivated rice (*Oryza sativa* L.) may have been domesticated from the wild progenitor (*O. rufipogon*) dated approximately 10,000-20,000 BP (Jiang and Liu, 2006; Wu *et al*., 2012). Based on morphological, biochemical, and molecular data, Asian cultivated rice is classified into two subspecies, i.e. *japonica* and *indica* (Oka and Morishima, 1982). In eco-geographical terms, the subspecies *indica* have grown throughout tropical Asia at low latitudes and low elevations, while the subspecies *japonica* are typically found in temperate East Asia, Southeast Asia, and South Asia at high latitudes and high elevations (Cai *et al*., 2007). Rice scientists now accept that rice was originally domesticated at the Yangtze River area in China (Gross and Zhao, 2014).

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Rice cultivation in mainland Southeast Asia rice appears by 2000 BC, especially associated with the lower reaches of the Red River (Vietnam), the Mekong River (Cambodia) and Chao Phraya (Thailand) (Fuller *et al*,. 2010). Based on fundamental questions of when rice cultivation began in Thailand archaeological evidence for rice cultivation have been studied at three archaeological sites (i.e., Pung Hung cave in the northern, Non Nok Tha in the northeastern, and Kok Phanom Di in the central region). It appears that around 1,800 to 2,500 years ago rice cultivation originated in Thailand (Bayard, 1970; Solheim, 1970; Watabe *et al.,* 1970).

Watanabe *et al.* (1963) reported grain morphology from rice husk contained in bricks of cultivated rice distributed in Thailand during four periods (6^{th}-11^{th}, 11^{th}-15^{th}, 15^{th}-18^{th}, and after the 18^{th} century). Grain types were classified into three types, i.e. round, slender, and large types. During the 6^{th}-11^{th} centuries (i.e., Dvaravati Period) in Thailand, rice grains with three types were found, with the round type as the dominant type. In this report, the round type is designated as "*japonica*" or "*japonica*-like" rice, while the large type of grains are of the upland variety and are presumably of the glutinous upland variety. For the slender type, most of the varieties were probably of the non-glutinous lowland variety. It is not unlikely that the round type belonged to the glutinous-lowland variety. This means that the rice grown since the 6th century was both glutinous (large type) and non-glutinous (slender type).

Oka and Chang first reported a *japonica* type of cultivated rice found in northern Thailand (Oka and Chang, 1963). These rice varieties consisted of both glutinous and non-glutinous rice. From the geographical distribution, it became apparent that the *indica* type was grown in lowlands, whereas the *japonica* type was found in high altitude areas (Vaughan *et al*., 2008).

Japonica is now subdivided into two subgroups called temperate *japonica* and tropical *japonica* or *javanica* (Oka, 1988). Temperate *japonica* rice varieties are distributed mainly in the islands of Western Pacific, from Indonesia to Japan, and in certain parts of the continent in China and Korea, while tropical *japonica* rice varieties are distributed on mountainous regions at low medium elevations in equatorial area (Oka and Chang, 1963). These two subspecies of Asian cultivated rice can be distinguished using classical diagnostic traits, including seedling resistance to $KClO_3$, seedling survival in cold temperatures, grain apiculus hair length, and phenol reaction (Morishima *et al*., 1992). The phenol reaction test showed that rice hulls and grains turn dark in color after exposure to a 1-2% aqueous phenol solution; *japonica* type showed no color change (or a negative response), whereas the hull of *indica* type showed positive response (i.e., a color change from yellow to dark brown or black) (Oka and Chang, 1961).

Dvaravati is a culture that the Dvaravati Culture were regarded as the first historic culture of present-day Thailand and has been roughly dated to c. 600-1,000 CE and it is clear that the Dvaravati Period marked a period of cultural growth, social complexity, and incipient urbanization ranging from locations such as Thung Sethi in the upper peninsula, north to Nakhon Pathom, U-Thong, and then across the central plains into the Korat Plateau in the northeast (Glover, 2011). Around 257-357 CE, the communities in the area of the Chao Phraya River developed the highest civilization in the region, with easy access to the sea and settling in a habitat in a river basin suitable for agriculture (Glover, 2011). Since 1968, no investigation had been conducted so far to identify which subspecies of these rice grains had been cultivated during the Dvaravati Period. It is therefore of particular interest to classify the Asian cultivated rice at the subspecies level. Archaeological evidences would address big questions about the past that in most cases cannot be ascertained as written records are absent or limited. This study allows us to formulate a tentative hypothesis that can be further explored and makes some general conclusions on the history of rice agriculture in Thailand.

Half a century of scientific research has reached no conclusions on the subspecies of cultivated rice which were domesticated in the Dvaravati Period of Thailand. It is proposed that the study of the morphology of rice grains through the comparison of grain morphology of old specimens with those of the present varieties or rice landraces will identify the specimens of each type and reconstruct the long history of rice-growing societies in Thailand. Thus, the objectives of the present study are to classify the grain morphology of rice hulls imprinted on bricks dated to the Dvaravati Period and to survey the phenol response in traditional rice landraces and then to determine whether the associations between grain morphology and the phenol response can be demonstrated in the two subspecies *indica* or *japonica*.

2. Materials and Methods

During 2014-2015, several surveys were conducted to search for bricks that contained molds (or imprint) of rice grains at selected historical sites in Thailand and Laos PDR. Some bricks were collected or their photographs taken from three historical sites of Thailand, all of which are registered at The Fine Arts Department: the Phrathatyaku (No.0003338, 16°19'12"N, 103°31'12"E) in Kalasin province; Umyaku (No. 0002909, 16°18'36"N, 103°18'00"E) in Mahasarakham province, both in the northeastern region; and Uthong ancient city (No. 0004914, 14°22'12"N, 99°53'24"E) in Suphanburi province, central region. In the Lao PDR, some bricks of the Old Vientiane Wall (17°54'05"N, 102°39'03"E), registered at the Department of Information and Culture in the Vientiane capital, were sampled and photographed. Figure 1 shows the specimens of imprints of rice husk on bricks from these historical sites. In addition, a collection of rice landraces from northern, central, northeastern regions of Thailand, Lao PDR, Vietnam and Cambodia were used to examine phenol reactions of rice hulls. Samples of 20 rice hulls found in a cave of an archeological site in Lao PDR were used to analyze grain shape and to examine the phenol reaction. Rice hull shapes (length and width and L/W ratio) were analyzed by using VISA Lab's A-VIS 4.0 (Amornsin, A. 2008. VISA Lab's AVIS Software, Mahasarakham University, Thailand, personal communication). In each of the historical site, 25 good shapes

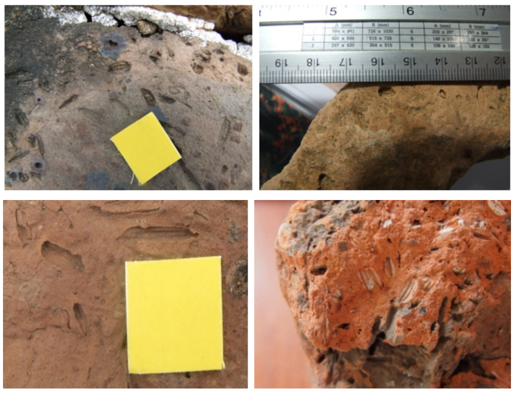

Figure 1. Example of imprints of rice husks on bricks from four historical sites dated to the Dvaravati Period.

of rice hulls imprinted on bricks and 20 rice hulls from a cave in a historical site in Lao PDR were selected to measure grain length and width. The ratios of length to width were calculated and grain shapes were then classified into round (short), large (medium) and slender (long) types, based on the methods formulated by the International Rice Research Institute (IRRI) (1996). The grain shape in terms of length to width ratio was determined using following equation:

Length to width ratio =
average grain length (mm)/average grain width (mm) (1)

where 1 is short (round) type of L/W ratio 2.2:1 or less, 2 is medium (large) type, 2.3:1 to 3.3:1, 3 is long (slender) type, 3.4:1 and more.

Furthermore, 259 rice landraces collected from Thailand, Laos and Vietnam were compared based on rice grain morphology. Rice grains were tested on phenol reaction and determined the endosperm type of each sample. For the phenol reaction, rice hulls were soaked in a 1.5% aqueous phenol solution for 24 hrs, dried and compared with untreated rice hulls of the same sample to determine color change. For starch staining, cross-sections of rice grains and starch granules were stained with an iodine solution (0.74 g of resublimated iodine and 1.48 g of KI dissolved in 400 mL of distilled water) and observed under a light microscope.

3. Results and Discussion

3.1 Rice grain morphology

Rice husks imprinted on bricks still preserved their individual characteristic showing their lemmas, paleas, and nerves (Figure 2). One hundred samples of complete imprinted rice husks in bricks were measured and were found that the length of rice husks ranged from 4.3 mm to 7.9 mm and the width from 1.8 mm to 3.4 mm. The length/width ratio of these rice husks examined ranged from 2.42 to 4.5 (Table 1). It is suggested that the rice husks examined may be divided into three types, i.e. round, large and slender types, each corresponding to *japonica*-like rice, *tropical japonica* and *indica*, respectively. For example, imprinted rice husks in Phrathatyaku exhibited three types of rice grains (Figure 1).

As suggested by Morinaga (1969), there are in Southeast Asia, *japonica* types that are identical with *japonica* in shape, round type, but different in characteristics. For example, the rice landrace named *kai-noi*, a glutinous rice from the northern part of Laos PDR exhibited the round type grain (L/W ratio of 1.53-1.6), and with a negative response of phenol reaction. This means that rice land race *kai-noi* is *japonica-like* rice.

Ahn (1993) suggested that L/W ratios are not affected by charring, therefore ancient and modern rice should be comparable. *Indica* rice normally has an L/W ratio above 2.5, whereas *japonica* rice is below 2.3 (Fuller *et al*., 2009). The present study, the length–width (L/W) ratios of the rice husks were compared with those of modern populations of domesticated rice. The majority of rice husks in bricks of Thai archaeological sites examined were classified as large type grains, with few slender and round types. These findings fit

Figure 2. Example of rice grain imprint on brick was measured for the Length/Width ratio by using VISA Lab's A-VIS 4.0.

Table 1. Length and width of imprint rice hull in bricks of four historical sites and rice hulls from a cave in Lao PDR.

Histological site	Number of rice hulls tested*	Length (mm) (range)	Width (mm) (range)	L/W (range)	Grain type*
Old city wall, Laos	25	7.9- 8.0	2.1-3.2	2.1-3.2	Round, Large
Phrathatyaku, Kalasin province,Thailand	50	8.5-4.5	2.2-4.5	2.1-4.5	Large Round Slender
Umyaku, Mahasarakham province,Thailand	25	7.8-6.5	2.5-3.1	2.5-3.2	Large
Uthong ancient city, Uthong province, Thailand	25	7.6-5.8	2.4-3.5	2.6-3.1	Large
Cave, Lao, PDR	20	7.25-9.8 (mean=8.8)	3.4-4.6 (mean=4)	1.7-2.4 (mean=2)	Round, Large

* according to IRRI (1996), Length/Width ratio divided into 3 types : 1) round type (L/W= 2.2 or less), 2) large type (L/W= 2.3-3.3), and 3) slender type (L/W =3.4 or more)

with the study of samples of grain shapes of 20 rice hulls found in a cave of an archeological site in Laos PDR. Table 1 showed that the L/W ratios vary from 2.5 to 3.2 (Umyaku), 2.6 to 3.1 (Uthong ancient) inferred that those rice examined were large type grains (or *indica* type). In addition, rice husks examined showed vary value of L/W ratio of 2.1 to 3.2 (Old city wall, Laos), 2.1 to 4.5 (Phrathatyaku) and 1.7 to 2.4 (Cave, Laos). According to Fuller *et al*. (2009), rice husks with L/W ratios below 2.3 belong to the *japonica* type.

3.2 Variations of phenol reaction and endosperm type of rice accessions in glutinous rice zones

Two accessions with large and round type of rice landrace from the northwestern region of Vietnam were stained with phenol solution. It was found that the large grain type showed glutinous endosperm and positive reaction of hulls (hulls turning black after staining), whereas, the round type rice showed non-glutinous endosperm and negative response to phenol reaction (Figure 3). These findings suggest that both glutinous and non-glutinous rice were cultivated by the local people in the area. The results of phenol reaction response of rice hulls also suggest that both *indica* and *japonica-like* rice were grown in this area as well. These results support previous reports on rice landraces classified into *indica* and *japonica* rice in other sites in China (Chen *et al*.,1994; Gross and Zhao, 2013), Bangladesh (Wang *et al*., 2013), and Vietnam (Fukuoka *et al*., 2006).

Based on the results of the phenol reaction of rice landraces in Thailand, the hulls of all upland rice landraces from the northern and northeastern regions of the country showed unchanged color of the hull when soaked in 1.5% phenol solution. In contrast, all lowland rice landraces (128 accessions) with different type of grains yielded a positive reaction, with the hull turning black. This finding further suggests that these rice landraces were *indica* rice. For example, the round type of a lowland rice landrace from northeastern region of Thailand named *pla-kheng*, and a rice land race with slender type grain named *hohm nangnuan* were classified as glutinous rice with a positive response to phenol reaction (i.e. *indica* rice). Today, most traditional rice landraces in Thailand and Laos with glutinous endosperm are large grain types (Prathepha, 2008; Phanthaboun, 2009).

Based on the study of isozyme and DNA, the cultivated rice varieties are classified into three subpopulations, i.e. *indica, temperate japonica* and *tropical japonica* (Garris *et al*., 2005). *Temperate japonica* varieties are known for their short, round grains and *indica* for their slender grains (Glaszmann, 1987). The comparison of grain morphology of old specimens with those of present rice landraces might allow inferring or better understanding the history of types of rice in this area (Figure 4).

Asian cultivated rice has adapted to different ecological conditions under both natural and human selections for the *indica* and *japonica* ecotypes (Chang, 1976), lowland and upland ecotypes and grain shapes (Glaszmann, 1987). Generally, upland rice has adapted to the water-limited and rain-fed rice ecosystems. On the other hand, lowland rice is commonly planted in fields with irrigation facilities. The findings of this study suggest that a type of grain shape of rice found in bricks do not identify into *indica* or *japonica-like* rice, because based on the results of phenol reaction response, both *japonica* or *indica* type showed variations of grain shapes (round, slender and large). For example, a round type of grain rice landrace in Laos (i.e., *kai noi*) and a large type of grain rice landrace (i.e. *hao ma fai*) (Figure 4) from northeastern region of Thailand, showed negative responses to phenol reaction. Therefore, the two subspecies (*indica* and *japonica*) of *O. sativa* grown in the Dvaravati Period in Thailand and Laos can be identified through the agro-ecosystem (i.e., upland rice or lowland rice). Based on the results of phenol reaction response of a collection of present rice landraces, it was found that upland rice landraces were classified as subspecies *japonica*, while lowland rice landraces were identified into subspecies *indica*. In this study, we propose that rice hull imprints found in bricks from the Dvaravati Period might belong to the subspecies *indica* and *japonica*. These findings support a hypothesis which was proposed by Somrith (1992) where imprints of hull of round-typed grain found at Ban Non Nok Tha, northeastern Thailand are more likely to be glutinous *indica* rice grown in flooded area, whereas the slender-typed (or *indica* type) and the large-typed (tropical *japonica*) were more likely grown in upland areas near the Pung Hung cave in the northern region of the country.

Figure 3. Rice hulls of cultivated rice (*O. sativa)* subspecies *indica* turning black after staining with phenol solution.

Figure 4. Comparison of three modern rice grain shapes, Kai-Noi, *tropical japonica rice* (round grain, left), KDML105 (*indica*, slender grain, middle) and Hao Maphai, upland rice (large grain, right).

4. Conclusions

Historically, Thailand, which was known as Suwarnabhumi, or the golden peninsula of Southeast Asia in ancient times, has cultivated rice through various historical periods from the Dvaravati, Srivijaya, Lopburi, Chiangsaen, Sukhotai, Ayudhaya through contemporary times (Chitrakorn and Somrith, 2003). The results of this study found that rice hulls in bricks from the Dvaravati Period could be classified according to the three types of cultivated rice grain shapes, i.e., round, large, and slender types. Findings from previous studies in archaeology, physiology, and genetics on the presence of domestication rice landraces suggest that the subspecies of cultivated rice at that time are of the *indica* and *japonica* subspecies (Katayama, 1995; Castillo, 2011). The findings of this study can now strengthen our understanding of the history of domestication of rice in this region.

Acknowledgements

This research project was funded by Mahasarakham University. The author is greatly thankful to the anonymous readers for their critical reading of the manuscript.

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