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Domestication and Long-Distance Dissemination of Rice: A Revised Version

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Abstract

Historically, in 1930s two sub-species, *indica* and *japonica*, were proposed on the basis of sterility in F1 hybrids between them. Subsequently, the two types were classified by associations of a number of genetically independent traits. The characteristic associations of traits have been explained by a series of hybrid sterility or reproductive barriers, in which a duplicate recessive gene model was proposed to cause varietal differentiation. In 1990s, most of hybrid sterility genes were proved to be caused by an interaction of allelic genes at each locus. In a well-known case of hybrid sterility, the *indica*, *japonica*, and wide-compatibility type which gives fertile hybrids in its cross to *indica* and *japonica* type, are proved to contain an allele, S5-i, S5-j and S5-n, respectively at a locus on Chromosome 6. Those gametes having S5-j allele is found to be partially aborted in the hybrid genotypes of S5-i/ S5-j while no gamete abortion occurred in S5-i/S5-n and S5-j/S5-n genotype. Since then, the S5-n has been used in hybrid rice breeding to obtain fertile and vigorous hybrids between *indica* and *japonica* types. For the last decade the action of S5-n is found to be due to a deletion in its gene sequence, while functional enzymes are produced by other alleles. Thus, the earlier assumed role of hybrid sterility genes as to lead to varietal differentiation are no more supported. Today, the characteristic association of traits found in each of varietal groups is better explained by founder effects. Recently , genetic analyses have been advanced for the contrasting traits between *indica* and *japonica* group. For an instance, genetic bases for long slender grain of *indica* and short wide grain of *japonica* are analyzed, and the short wide type is found to be caused by a deletion in its gene sequence. Thus, the short wide grain is considered to be mutational origin during domestication. On the other hand, a large number of native cultivars of rice were surveyed with enzyme polymorphism in 1980s, and later with molecular markers. As the results, profound genetic diversity is found in the genetic background of rice and wild rice. As the results, some scientist proposed the idea of multiple independent domestications of rice. However, before reaching to such a conclusion, at least two factors, i.e., long-distance-dissemination of some genotypes and possibilities of introgression by local wild rice to primitive cultivars need to be examined. Taking the two factors as well as historical events into consideration, the author considered that perennial *japonica* cultivars were first domesticated in Changjiang river basin, and disseminated to East India through Assam or along Bengal Bay, meanwhile their genetic background were transformed under introgressions of local wild rice which resulted to form the *indica* types. Thus–formed *indica* types seem to be disseminated to Southeast Asia, for an instance to Champa, under the expansion of Hinduism. Later some of the genotypes were introduced into China. Recently, this explanation has been verified by extensive analyses of gene sequence for wild and cultivated rice.

Keywords: Hybrid sterility; Reproductive barriers; Varietal differentiation; Genetic diversity; Geographical distribution; Founder effect; Transgression of wild rice; Domestication

Introduction

The center of domestication of rice has been assumed to be in tropical or subtropical regions. *Indica* type (Hsien) has been considered to be an ancestral type from which *japonica* type (Keng) and *javanica* types or tropical *japonica* types were differentiated [1]. But the earlier view on the origin of cultivated rice has to be changed by a number of reasons. First, archeological findings of the oldest rice cultivation provided evidence that rice must have been domesticated in the mid and lower Changjiang river basin. Second, wild rice populations have been discovered in the same region by extensive surveys in 1980s [2]. Third, a large number of local cultivars have been surveyed by enzyme polymorphism since 1980s, and recently by molecular markers. As the results a profound genetic diversity has been found among different groups. The new results suggest the process of domestication might have been much complex than that conceived earlier.

Today a re-construction of earlier concepts seems to be necessary to understand a consistent picture of distribution and domestication of cultivated rice. Initially, the author published an extensive review in other paper [3]. Since then, there are some important research results. Therefore, while avoiding details of the early version, here above mentioned three points are to be detailed in the light of recent progress.

Earlier Concepts for *Indica-Japonica* Differentiation in Rice

It has long been recognized that there are two types of rice, ‘Hsien’ in the South and ‘Keng‘ in the temperate or northern region in China. The two types were first proposed as two sub-species, namely *indica* and *japonica*, by Kato et al. [4], who found varying degrees of spikelet sterility in F1 hybrids between the two types. By the time Kato distinguished the two types on the basis of hybrid sterility, a general understanding had been formulated that the sterility of inter-specific hybrids is caused by failure of chromosome paring at the reductive cell division in F1 hybrids due to structural differences between parental chromosomes. Following that idea some scientist interpreted the level of hybrid sterility as a criterion to see the degree of differentiation at the chromosomal level, and conceived the hybrid sterility as a measure of taxonomic distances.

After the initial taxonomical works, the *indica* and *japonica* groups were classified by associations of a number of characteristics within each group [5]. For an instance, long grain and less sticky rice after cooking are commonly found in *indica* types, while short grain and sticky rice are in *japonica* types.

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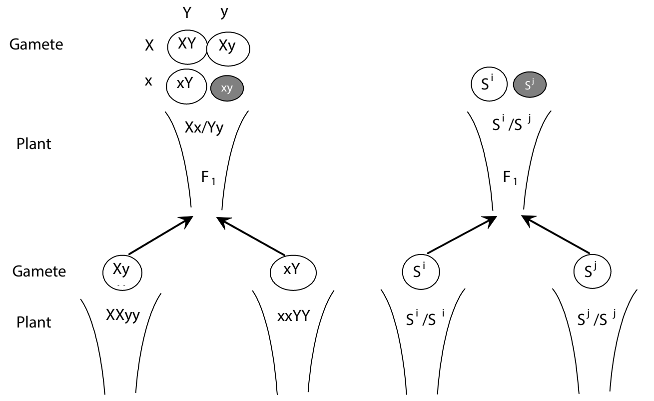
Terminology for *Javanica* or Tropical *Japonica* type.

In addition to *indica* and *japonica* types, another term '*javanica* type' or tropical *japonica* type has been introduced. The term of *javanica* was first proposed by Morinaga [6] on the basis of an extensive survey of Asian rice cultivars in Japan by Matsuo [7] who first classified them into three groups, A, B and C, of which a distinct varietal group B was considered to have originated in Java while A and C were considered to be *japonica* and *indica* type, respectively. Oka and Chang [8] further classified the *japonica* into two subdivisions, namely, tropical *japonica* type and temperate *japonica* type, and these terms have been popularly used.

Explanation of the *indica-japonica* differentiation

Oka proposed an idea that the observed characteristic associations of various traits in *indica* and *japonica* type may have arisen by a mechanism of reproductive barrier which reveals partial hybrid sterility [9]. He emphasized the importance of partial sterility in F1 hybrids between *indica-japonica* type, and attributed its genetic basis to a set of duplicate gametophytic sterility genes. Here, the detail of his explanation is not repeated, as it was given elsewhere [9,11], but his conclusion can be cited as follows. Oka considered there are many pairs of such 'duplicate recessive lethal genes' in the rice genome (Figure 1). Thus, the characteristic associations of genetic traits in *indica* or *japonica* type were explained on the basis of genetic mechanism for reproductive barriers. Further, the differentiation of rice into *indica* and *japonica* groups was explained on the basis of such reproductive barriers, which are interpreted as an inner genetic mechanism to lead the differentiation.

Despite the lack of firm experimental evidence Oka's genetic model [11] has long been accepted, because it explained the hybrid sterility and characteristic association of traits among different groups of rice as well as their differentiations. Oka himself has repeated his idea until he passed away. He summarized the idea as follows: 'In hybrids, genes tend to be associated in a certain manner across independent loci', and that 'this can be partly elucidated by the presence of many sets of duplicate or complementary genes for gametophytic and sporophytic sterilities, and seems to suggest an internal mechanism of genetic differentiation, which could be a complementary system of adaptive gene blocks' [12].



Those gamete genotype in shadowed circle denote partially or completely aborted ones

Figure 1: Genetic Model for Hybrid Sterility. Two locus model (left) and one locus model (right). In the two locus model, two isolated genotypes contain the gametophytic lethal gene genotype of *XXyy* and *xxYY*, respectively. The genotype of hybrid between them should be *XxYy*, from which four gamete genotypes will be produced after the meiosis, i.e., *XY, Xy, xY* and *xy*. Gametes with the two recessive genes (*xy*) will be aborted on the F_1 hybrid before fertilization, and lead to partial sterility of panicle or pollen in anther. In the one locus model, those gametes having *S-j* gene are partially aborted in the hybrid genotypes *S-i/ S-j.*

A New Understanding of Hybrid Sterility Genes, and their Origin and Function

The present author did have a chance to test Oka's assumption in early 1980s. After identifying some varieties which showed 'no hybrid sterility in their crosses to *indica* and *japonica* testers, the author named this character as 'Wide Compatibility (WC)' for convenience, and analyzed its genetic nature.

Hybrid sterility by allelic interaction at a locus

It was found that *indica*, *japonica* and the WC type contains single allele, respectively, S5-i, S5-j and S5-n at a locus on the short arm of Chromosome 6. And those female gametes having S5-j gene are partially aborted in the hybrid genotypes S5-i/ S5-j as shown in the right side of Figure 1, while no gamete abortion occurred in S5-i/S5-n and S5-j/S5-n genotype [13,14].

After testing of about one thousand varieties in China, the hybrid sterility in Chinese cultivars was found to be caused mostly by the allelic interaction at S5 locus. A few varieties showed hybrid sterility in their crosses to Wide Compatibility Varieties (WCV) [15]. In a wide range of varietal testing a few varieties from Indian Subcontinent also exhibited hybrid sterility when crossed to WCVs. Then, further genetic analyses of hybrid sterility gene loci (HSGLi) were conducted following the identification of S5 locus. As the results, some more loci following the same genetic model were identified on different chromosomes in other hybrids [16]. Studies to identify HSGLi have been continued until now [17].

Application of wide-compatibility gene to hybrid rice breeding.

As soon as the simple monogenic nature of hybrid sterility was understood, it was applied to hybrid rice breeding to enhance the level of hybrid vigor. Since early 1990s the S5-n has been incorporated to various rice cultivars to obtain fertile hybrids in hybrid rice breeding, because the hybrid sterility was overcome by the incorporation of S5-n allele into *indica* or *japonica* type. In practice, it has been found that the hybrids between *indica* type and *javanica* type are more productive in many areas than those between *indica* and *japonica* types.

Initially, the hybrid sterility alleles were confirmed by female gamete abortion in embryo sac, as it is easily detectable by partial sterility in panicles, but the same genetic mechanism for male game abortion has been described. When *indica -japonica* hybrids were first released in China, some of the hybrids were extremely sensitive to cold temperature due to a high level of pollen sterility, which was found to be caused by the hybrid sterility, as it is for female gametes. Accordingly, a set of neutral alleles for such a hybrid sterility in male gametes were studied and applied to obtain a sound pool of pollen in inter-subspecific hybrids [18,19].

Molecular analyses of hybrid sterility genes

The role of hybrid sterility genes at molecular levels seems to be diverse. They constitute a part of genes for normal gamete formation, and are detected by a partial gamete abortion in *indica-japonica* hybrid due to an interaction between different types of protein. The S5 region has been mapped [20] and covers up to five open reading frames (ORF1 to ORF5). Transformation studies of ORF3 to ORF5 from an *indica* variety into a *japonica* variety showed reduced fertility, due to embryo-sac abortion, for transformants harboring *indica* ORF5, whereas the fertility of transformants of ORF3 and ORF4 was not affected. The *indica* and *japonica* alleles of ORF5, which encodes an aspartic protease, differ by two nucleotides, whereas the wide compatibility allele has a large deletion in the N terminus of the predicted protein, causing subcellular mislocalization of the protein.

It is noteworthy that a case of mutational change of a hybrid sterility allele was found in an experimental line, 02428 from China which possesses the S5-n allele. The parents for 02428, Pangxiegu and Jibangdao, were proved to possess S5-j. The neutral allele S5-n in 02428 was considered to be induced from S5-j by irradiation of 60Co to the parents [21].

Initially the author considered that the Wide Compatibility Genotype (WC) is an ancestral type and the *indica* or *japonica* type is produced by defects in their gene sequence. It was assumed that such a neutral allele as S5-n should be original allele having been conserved in an open-pollinating population while other alleles like S5-i and S5-j might have been lost in an open pollinating population due to its disadvantage of gamete abortion. But, since the WC is produced by a deletion in the gene sequence, the author's initial assumption cannot be supported.

After molecular analyses of some other hybrid sterility genes, it was found that the mechanism to reveal gamete abortion is much diverse and involves complex interactions of proteins which are related to a pathway to programed cell death.

Even after the analyses of hybrid sterility genes at molecular level, a group of scientists are attempting to explain the differentiation between *indica* and *japonica* rice by the action of hybrid sterility genes as a major source of genetic diversity in the rice gene pool. The author here would not detail their exhaustive studies. As discussed later in this paper the origin of *indica-japonica* differentiation can be explained by other way of consideration.

Founder effect for the association of traits.

As explained above the hybrid sterility in rice has long been assumed to be major reproductive barriers, which have caused isolation of varietal groups and led the crop into differentiation. Oka, as explained above, ascribed the association of characters in respective varietal group to the role of duplicate recessive genes. There are still a few idea to relate such reproductive barriers to the varietal differentiation, but the characteristic associations of traits in variety groups can be easily understood by selection and propagation of a few source genotypes or a founder to a wide area or distant regions. The domestication of rice is essentially propagations of such genotypes as found better than others, and multiplication of them by farmers via seed or vegetative stocks. If a set of traits are possessed by a pool of ancestor plants, the set of traits will be largely conserved by its progeny plants retaining the characteristic combination of the traits (Figure 2). Thus, the founder effect seems to be a very important mechanism to form a varietal group in domestication or dissemination. There is no room for any sterility gene to contribute to the association of characters.

Distribution of Rice Genotypes Examined by Enzyme Polymorphism and Molecular Markers

So far the relatively clear separation of *indica* type and *japonica* type has been observed in mainland China and its vicinity. By surveying isozyme variation for a large number of cultivated varieties Glaszmann [22] has shown that there are predominantly *indica* type and *japonica* type in the East Asia while there are a set of intermediate types from Burma to the Indian subcontinent. Having observed the contrasting pictures of distribution of rice genotypes, i.e., the region with two major types and the region from Myanmar to Indian subcontinent with various intermediate types, he indicated that there are two alternative gene pools, one in the East Asia and another in Indian subcontinent. It is noteworthy that the similar tendency has been observed in the picture of variation for the hybrid sterility loci.

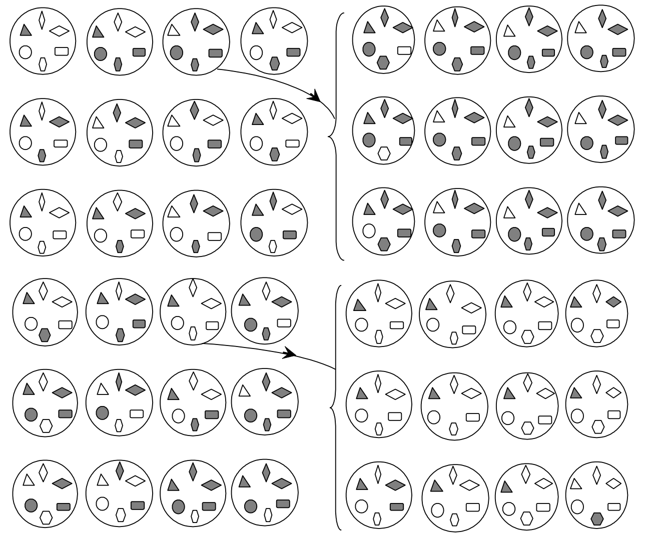


Figure 2: Association of traits via selection and distribution by human activity. Each circle denotes a cultivar, and six marks within it are traits. By selection and multiplication of some particular types association of traits would have been formulated. The left side shows diverse types conserved in earlier cultivation in which genetic diversity of wild types were retained, while the right side shows two predominant types as found in East Asia.

Indica-Japonica differentiation found with the use of molecular markers

It is interesting to see whether above-mentioned pictures of differentiation of rice are shown or not with molecular markers since the late of 1990s. One approach to describe genetic diversity of varietal group is to compare differential sequences in the vicinity of known genes. Londo et al. [23] studied three gene regions of 203 cultivars of O. sativa and 129 populations of *O. rufipogon*. They classified such haplotypes as A for wild rice in Thailand and India, B and C for *japonica*s and *javanica*s, of which B seemed to closely correspond to *javanica*s. The haplotypes D and E are found in *indica* rice and in some wild rice in india and indochina, of which haplotype D are found in india and Malaysia suggesting the domestication of Aus varieties in India and Malaysia. They concluded that the two major haplotypes of cultivated rice i.e., *japonica* and *indica* were domesticated independently from wild rice gene pools from southern China and India/Indochina, respectively. In a similar approach Zhu and Ge [24] analyzed introns of four nuclear single copy genes on different chromosomes for 37 accessions representing two cultivated and six wild species. Two subspecies of *Oryza sativa* ssp. *indica* and ssp. *japonica* formed two separate monophyletic groups, suggestive of their polyphyletic origin. It is reasonable that the pictures obtained earlier by Glaszmann with isozymes and those by molecular markers seem to be similar, because the materials in the test were derived from the similar source.

As an another approach to measure degrees of divergence, LTR(long terminal repeat) transposable elements have been used to see the divergence of varietal groups, because at the time of insertion the two LTRs of a given element were identical in the sequence but would have differed due to accumulated mutations. The extent of divergence may be proportional to the time elapsed since the insertion. Ma and Bennetzen [25] surveyed the divergence of genomes of *indica* and *japonica* types. The genome sizes of both *indica* and *japonica* have substantially increased since their divergence from a common ancestor mainly because of amplification of LTR-transposons. The sequences of genes were observed to have a very high rate of divergence. By comparing the genomic divergence with that observed for *Oryza galaberrima* as a reference genome, the divergence of *Indica* and *Japonica* is considered to date back long before the domestication of these two types. Vitte et al. [26] compared 110 LTR retrotansposons in the published DNA genome sequence of Nipponbare and 93-11 (an *Indica* type), and found that the two types diverged from one another at least 200,000years ago, older than the date of domestication. They also applied the insertion polymorphism to a wide range of traditional rice varieties of both *indica* and *japonica* types, and found that they arose from two independent domestication events in Asia.

Genetic diversity of wild rice as the source for heterogeneity of cultivated rice

Even within a few sample populations of wild rice, it is shown that there are diversified 'allelic differences' far beyond the range of *Indica* or *Japonica* type. Genetic diversity of 16 vegetative strains of wild rice (*O. rufipogon*) from three regions of Myanmar was evaluated by a genome-wide survey with SSR (single sequence repeat) markers [27]. Allelic diversity among the wild rice genome was evaluated by primer pairs each of which was assigned to 74 loci over 12 chromosomes of the genome. The loci in the wild rice genome revealed a large number of specific alleles in much wider ranges than those detected in six cultivars of O. sativa. Around 50-60 percent of alleles are specific to wild rice, while those identical to *indica* or *japonica* alleles, or common to both the cultivated and wild rice are only 10-15 percent of the total allelic diversity (Figure 3). It is noteworthy that the vegetatively propagated clones of wild rice contain such diversity.

The observation of diversity of wild rice population may lead us to an idea that only a part of their genetic diversity have been taken in the course of domestication of rice and that the domestication itself may not be the reason for the diversification of rice.

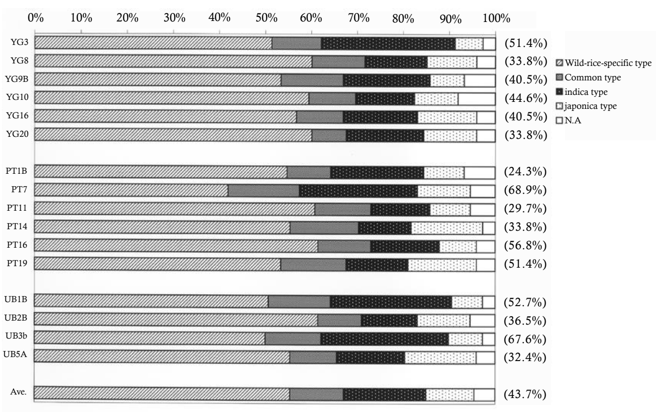


Figure 3: Evaluation of genetic diversity of 16 strains of wild rice (*Oryza rufipogon* Griff.)collected in three regions, Myanmar using using simple sequence repeats (SSRs).

Domestication of rice in the light of archaeological evidence

The genetic divergence of *indica* and *japonica* types has been supported by isozymes, RFLP, transposon insertions. All of these studies showed a clear genetic differentiation between the two varietal groups, and seem to lead to a set of independent domestication of the two types. The diversity and distribution of existing native cultivars are often cited as the evidence for multiple domestications. But, that would be correct only if there were no long-distance-disseminations of genotypes by human activity and no introgression of local wild rice into primitive cultivars. To re-examine the ideas of single or double domestication hypothesis for rice we may have to see some historical and cultural aspects.

Two key findings in China

Since 1970s archaeological evidences have indicated that rice must have been domesticated in the mid or lower Changjiang river basin. Following the discoveries, by an extensive survey [2], a wild rice population is found at a shallow swamp in the same region at northern latitude of 35°in Jiangxi province. Another wild rice population is found in "t'u ling" county in the south west of Hunan province, China. In the two sites perennial populations of rice are regenerating via vegetative stems every summer after freezing winter cold.

Genetics of some contrasting traits in rice

Perennial versus annual habit of growth: The cultivated rice in the temperate zone is potentially perennial. It is seeded in spring and harvested in autumn, but keeps an ability to regenerate its stem and flower again in next season when climatic conditions allow it. In this sense the cultivated rice in temperate zones is direct descendants from perennial wild rice in Changjiang river basin. The perennial habit has been inscribed through the process of domestication, as many groups of primitive rice must have been propagated in the time of domestication by dividing and transplanting of sprouting stems in early summer in shallow marsh, which was nothing but the origin of transplanting cultivation of rice [28]. In that process, early types with bigger panicles and weak seed dormancy might have been selected and converted into 'cultivated types'. Contrastingly, rice genotypes in Indian subcontinent and improved *Indica* cultivars are predominantly annual. According to a QTL analysis of *indica-japonica* hybrids, the annual type or those with earlier senescence are genetically recessive [29]. Therefore, the annual type may have arisen from the perennial type through a set of recessive mutations.

Short versus long grain and the mutation origin of short type: The long slender (LS) versus short wide(SW) grain have long been cited as one of most contrasting characters between *indica* and *japonica* type. The author has searched for as many reports as possible to see any description of wild rice with SW grain, but never encountered such a report. Then, I came to consider that the SW grain might have been originated from mutations after domestication. In our earlier study [30] the quantitative locus (QTL) for the contrasting trait was analyzed by comparing two back-crossed populations, i.e., IR36/NK2//NK2 and IR36/NK2//IR36, in which NK is a *japonica* type with SW grain and IR36 is an *indica* type with LS grain (Figure 4A and 4B). In the result, the most effective QTL was confirmed only in the population of IR36/NK2//NK2 (Figure 4), and the difference of grain width was not detected in IR36/NK2//IR36. The result *indica*ted that SW grain

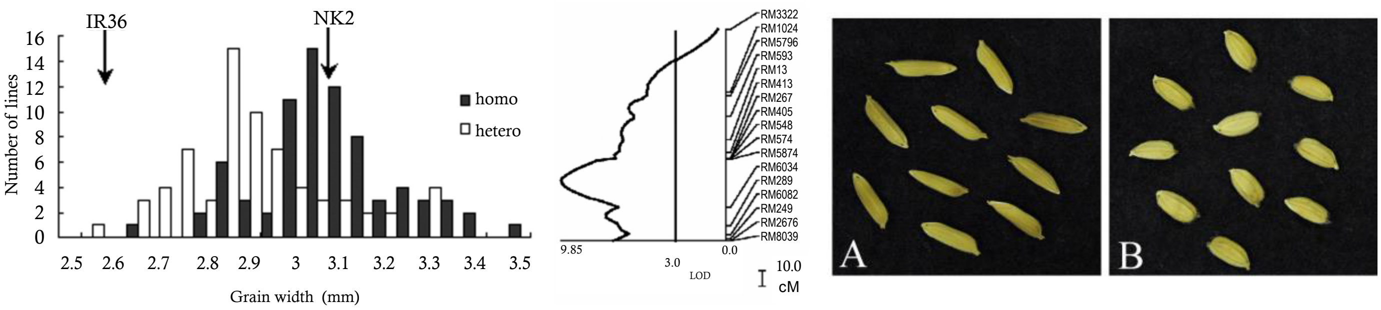


Figure 4: Genetic analysis for grain shape. Left: The QTL for short wide grain was detected only in the heterozygotes in IR36/NK2//NK2, whereas no difference between the two genotypes was detected in IR36/NK2//IR36. Center: The QTL for grain width on Chromosome 5. Right: A: IR36 with long slender grain, B: NK2 with short wide grain.

of *japonica* type is genetically recessive and revealed in the recessive homozygotes (Figure 4 (left)). Later a group of scientists analyzed the gene sequence of this QTL, and found that the SW type is caused by a deletion in the functional nucleotide polymorphisms [31]. The change of grain shape is related to a level of yield increase [31]. They considered that grain shape might have been selected as a favorable trait by ancient humans during rice domestication. But the selection toward SW grain type may not have been easy for ancient farmers, because so far all the grain samples excavated from archeological study in China are more or less a mixture of LS and SW type as indicated later in this review.

Differentiated farming culture in India from East Asia

To discuss the possibility of independent domestication of rice in India, first, we have to consider the role and characteristics of rice cultivation in India and East Asia. In East Asia we are able to see much diversified and elaborated use of rice products including sticky or glutinous rice, rice wine, and etc., while rice is less important and its use is often similar to other cereals in India. If rice had been originally domesticated in India, there should have been unique or original usage of rice and its byproducts. When we see a poor rice-based cultural complex in india, rice may have been brought there from Assam or Yunnan province of China.

Second, it should be considered that the present style of farming in India was introduced by the people who had migrated to India with cattle, cattle-drawn carts and upland cultivation of cereal crops. For them perhaps there was no need to domesticate any indigenous wild plants like wild rice in marshes.

Rice in tuber crop-based farming in the south-east Asia

As the plains in the Indian subcontinent are today dominated by the cattle-drawn farming, it is difficult to see any primitive cultivation of cereal crops before their migration to India. Only through the life styles of minority groups in hilly areas to which cattle-drawn carts did not reach, it is possible to see some sorts of traditional cultivation that were inherited by native people and common from Shan states in Burma to the western end of Assam highland. There are dominant remains of traditional tuber crops in the eastern parts of India, like banana, sugar cane and turmeric, which had been domesticated in Southeast Asia under a root-crop-based agricultural system. As discussed by Sauer [32], rice might have been also domesticated through vegetative propagation in the same system and been a component in such an earliest agriculture, whereas the present cereal agriculture in India could have been brought by a series of migration from elsewhere not earlier than BC 2000 or so.

Thus, rice must have been introduced into India by minority peoples from initially domesticated areas through Assam highland or the coastal zone along Bengal Bay. The independent domestication of rice in Indian subcontinent seems to be unlikely. Whereas, the very ancient remains of rice cultivation are found in the mid and lower basin of Changjiang river, where rice was primarily domesticated.

How did the Cultivated Rice Acquired their Genetic Diversity

There are a large number of traits which are characteristic to so-called *indica* and *japonica* type, out of which some can be attributed to peoples' preference or to an agronomic importance. The *indica-japonica* difference is not only relating to practically important traits but also covers the entire genome, as detected by molecular markers. How was the difference in the entire genetic background introduced or retained into cultivated rice ?

To answer this question, we have to think of interference by wild rice populations to cultivated rice in its course of dissemination. In that course perhaps major qualities essential to cultivation must have been conserved, but wild rice in the way of dissemination of cultivated types must have contributed to re-building or modification of genetic background of cultivated types through occasional hybridizations or introgressive pollination. Even today, it is not rare that wild rice populations as weed overwhelm the cultivated rice in Assam region of India (personal communication in Assam).

Implication of nuclear-substituted types in Assam

It is interesting that a substantial number of cultivated rice in Assam region are found to keep cytoplasma of *japonica* type while keeping a set of nuclear genes of *indica* type. According to Kaneda et al. [33] out of 51 native cultivars from Assam twelve showed nuclear genes of *Indica* type under the cytoplasm of *Japonica* type. The fact that a substantial number of rice cultivars in Assam region contained *japonica* type cytoplasm while showing a genetic background of *indica* type may imply a process of introgressive hybridization, which can be explained as follows.

The *japonica* type of rice has perennial habit, which could have been vegetatively propagated by transplanting in primitive cultivation in Changjiang river basin in China [3]. That type may have been introduced as a component of tuber crop-based-farming from its original region into Indian subcontinent through Assam highland as explained before. The *Japonica* type may have been pollinated by wild rice around farms and thus-formed seed was dropped around the mother plant. The progeny of such plants may have been selected to restore a set of characters for cultivated rice for some generations, but repeatedly been pollinated by surrounding wild rice plants which contain a different genetic background that is today recognized as the background of *indica* type. Such pollination by wild rice could have been repeated with a long interval during which a set of wild rice-affected traits would be selected out, thus, the cultivated rice must have been converted to *indica* likes in its genetic background while maintaining cytoplasm of *Japonica* rice together with basic characters as cultivated rice.

Introgression by wild rice as a differential genetic background of cultivated types

The nuclear-substituted types may be an extreme case of introgressive transformation, but suggest a frequent introgressive pollination by surrounding wild types which contain a vast range of genetic diversity. Such an introgressive pollinations may explain a reasonable origin of cultivated rice with the diversity of *indica* type. As the cultivated rice was hardly isolated from wild rice except for the area where no wild rice existed, it is rather unusual to conceive that the cultivated rice was expanded into various parts of Asia without any modification by local wild rice populations. The initially domesticated types in China could have been reformed by such an extensive transgression by local wild rice populations in its dissemination through Assam highland or along Bengal Bay to East India, where the transformed rice may have constituted the secondary center for divergence under the influence of traditional Indian way of cereal cultivation. To confirm this hypothesis further it is necessary to apply molecular markers to existing local cultivars and wild rice. So far the possibilities of transgression by wild rice do not seem to be carefully examined. Only recently, however, this aspect has been surveyed as introduced later in this review.

Distribution of *indica* and *japonica* Type under Human Activity

In the discussion of *indica-japonica* differentiation there are two aspects, i.e., one is differentiated genetic background which can be found through isozyme polymorphism or on the basis of molecular markers, and another is a series of agronomic or practically important traits like grain shape and cooking quality. The difference in terms of genetic background is shown today clearly with molecular markers, and understanding of this aspect is leading to the idea of independent origin of *indica* and *japonica* types. But the idea of the independent domestication of rice outside of the Changjiang river basin is not consistent with the historical and cultural background as discussed above. Thus, the author attempted to ascribe the genetic diversity of varietal groups to the introgressive pollination of wild rice in the course of dissemination.

Long-distance-dissemination of long grain types in China

The long grain types (hsien) with less sticky quality have been prevalent in southern region from Yangze river basin, while the short grain types (keng) in the northern regions from Huai Ha river basin. The two types were believed to have differentiated on an unknown reason and distributed in the South and North, respectively.

When we see ancient rice grains excavated in archaeological sites, it is interesting that grain samples excavated in Guangxi province in the southern border of China are round-shaped together with some intermediate type, which is dated back to 2,000 BC. Also, many grain samples from ancient remains are a mixture of long slender and short wide shaped together with some intermediate type, from the lower Changjiang river basin to Zhejiang and Hunan Province (Figure 5).

Therefore, it is rather reasonable to assume that the grain shape has been artificially selected toward a short round type from the original long type of wild rice under the preference to the round shape, which tends to give a higher milling recovery for cooking in granular shape. Those excavated from ancient archeological sites must have been domesticated in China and constituted the keng or *japonica* groups of rice.

On the other hand, the existence of long grain types with a genetic background of *indica* types in the southern region in China can reasonably be explained, if we assume that they might have been introduced from somewhere into southern region of China. Here, our attention should be called to a series of historical documents. First, in 1012 the Emperor Zhen Zong of Song Dynasty (in North) issued a decree to introduce a large quantity of seed of Champa rice seed from Fujian Province to Jiangsu, Zhejiang, Jiangxi [34]. Champa was in the mid of Vietnam where a Hindu kingdom had once been built. The background of the policy in 1012 was an urgent need to promote rice production in the granary from Changjiang river basin to the southern region, when the Yellow river basin had been threatened by a growing invasion of nomadic peoples from North and a great number of people had to migrate to the South. In 1192, it was recorded that the Champa rice was planted to 80-90% of the lower Changjiang river basin [34]. That type tends to vigorously thrive in a high water temperature than those in the temperate zone, so that presumably the Champa rice would have been more productive than local traditional ones. The prevalence of long grain rice in southern China in Song Dynasty is also noticeable in Japan to which long grain rice had been introduced sometime since the 13-14th century, where traditionally round grain rice had been prevalent.

It is also important to understand that the Champa was one of the kingdoms established under cultural and political influence of Hinduism from East India which could have been a secondary center of rice diversification as explained above. In India a number of agronomic characters seem to be altered. For instance, the shape of rice grain had not been converted into short or round under the preference to cooking by flour. The annual type of rice might have been preferred in an advanced upland cultivation with cattle-drawn plows, in which annual types do not hinder land preparation with surviving stocks. The typical *indica* type of rice as detected in Glaszman's work in East Asia, is composed of a dominant type, which may have been selectively introduced to Southeast Asia out of many intermediate types in the secondary center in India and provided a founder effect.

Another case of long-distance-dissemination

The importance of human activity for varietal characteristic as indicated above can also explain the extension of tropical *japonica* or *javanica* type into hilly areas of Southeast Asia through Indochina peninsular to Java and Philippine archipelago. This type of rice which is characterized by traits of upland rice like as thick stems with deep root might have been extended to the South by rice-cultivating tribes in hilly areas from China before the *indica* type was widely introduced into this region (Figure 5). If both *indica* and *japonica* types had been mixed in the southern China in ancient times, as often understood by archeologists, both types should have been brought to southern regions from the center of domestication. The fact that those similar to *japonica* type had been extended to the South implies that those which were domesticated earlier in the Changjiang river basin were *japonica* type and that the *indica* type would have been introduced later as discussed above. The entire picture of development and distribution of rice genotypes throughout Asia can thus be illustrated by Figure 6.

Figure 5: Carbonated grain samples excavated from one of the earliest archeological sites in t'ien lo shan ' near a famous archeological site, Hemudu, Zhejiang Province, China. The grain samples contain various types of grain including long slender and short wide. The excavated site seemed to be earlier than the era of Hemudu site (ca. BC 5000). Photo was taken by the author via the courtesy of Dr. Zheng Yunfei, Zhejian Provincial Institute of Cultural Relics and Archeology.

Verification of the hybrid origin of *indica* type

The hybrid origin of *indica* type of rice was first proposed as mentioned above by the author. Recently, it is most interesting that such an explanation is verified by an extensive survey of DNA sequence of cultivated and wild rice by Xuehui Huang et al. [35]. They generated genome sequences from 446 geographically diverse accessions of the wild rice species Oryza rufipogon and from 1,083 cultivated *indica* and *japonica* varieties to construct a comprehensive map of rice genome variation. The most persuading approach in their method is the identification of 55 gene sequences for such traits as advantageous in cultivation and assumed to have occurred during domestication. These gene sequences were proved to be commonly derived from a group of *Oryza sativa japonica* rice and conserved among all the cultivated rice. Then, they concluded that the first domesticated is from a specific population of *O. rufipogon* around the middle area of the Pearl River in southern China, and that *Oryza sativa indica* rice was subsequently developed from crosses between *japonica* rice and local wild rice as the initial cultivars spread into South East and South Asia. Their conclusion verifies the author's earlier view shown in Figure 6, although whether or not the initial domestication was from a population in the Pearl River basin seems to be a question, because most of ancient wild rice had been wiped out in China, and is not available for such analyses except for a few.

Conclusion

Two sub-species, *indica* and *japonica*, which are characterized by associations of a number of genetically independent traits, were long assumed to be differentiated through reproductive barriers or hybrid sterility. Since 1980s, the hybrid sterility has been found to be caused mostly by an independent allelic interaction at some loci. At the same time at each locus for the hybrid sterility, a neutral allele which does not cause hybrid sterility in *indica-japonica* hybrids has been found and applied to produce *indica-japonica* hybrid rice. On the other hand, the distributions of rice genotypes have been surveyed with enzyme polymorphism in 1980s and recently with molecular markers. In the light of recent studies, first, the action of each set of hybrid sterility genes are diverse at a molecular level, and not readily associated to the varietal differentiation. The characteristic association of traits in each group of rice can reasonably be explained by founder effects, as some limited types can be widely propagated by human activities. Second, the outstanding contrast of grain shape, i.e., long slender and short wide grain has been genetically analyzed. Then the contrast was found to be caused by a deletion in the original gene sequence for long grain in the course of domestication. Third, considering historical events, the author considered that perennial *japonica* cultivars which is close to wild rice in terms of perennial habit first domesticated in Changjiang river basin were disseminated to East India through Assam or along Bengal Bay. There they were transformed under occasional introgressions of local wild rice and formed a secondary center from which some genotypes seem to be disseminated to colonies under the influence of Hinduism in Southeast Asia. Later some of the genotypes were introduced into China. This is the answer to the question of *indica-japonica* differentiation. Recently, this earlier view is verified by an extensive survey of DNA sequence of wild and cultivated rice of the world.

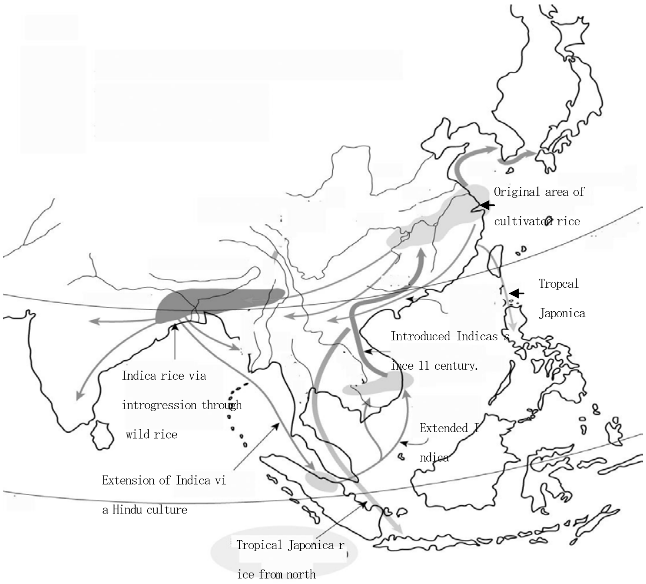


Figure 6: Domestication of Japonica–type rice in Yangze river basin, and its extension by human activity and historical events.

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