Phylogenetic Relationship and Classification of *Vigna radiata-mungo* Complex

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Vigna radiata-mungo complex is a group of edible legumes originated from tropical Asia, which is composed of mungbean (*Vigna radiata* (L.) Wilczek), black gram (*V. mungo* (L.) Hepper) and related wild forms. These legumes are consumed not only in the form of sprouting bean, but also in the form of boiled bean and boiled bean-powder. They play an important role as a dietary protein source in tropical Asian countries. In Japan, too, 50–60 thousand tons of *V. radiata-mungo* complex is imported annually, and mostly used for making bean sprout.

Like many other tropical legumes, the production technology of these legumes has not been improved, and their yields remain at a low level.[11] In view of the continuous food shortage in tropical Asian countries, it is important to increase yields of these legumes by improving varieties and cultural techniques. However, regarding their classification and phylogenetic relationship, which are regarded as a basis for researches and experiments for that purpose, there are many contradictory reports.[1,10,12,13,16] This situation is becoming an obstacle in promoting efficiently the varietal improvement of mungbean and black gram.

The taxonomic confusion seems to be caused by morphological similarities between mungbean and black gram, and remarkable morphological variations among the wild forms. Recently, *V. radiata-mungo* complex was rearranged by Verdcourt[16] as composed of *V. radiata*, *V. mungo*, and *V. radiata* var. *sublobata*, which includes all wild forms. However, it was found out later that there was the differentiation of lines, which appear to be corresponding to *V. radiata* or *V. mungo*, from the wild forms.[2,3,6,7]

The authors attempted to examine morphological characteristics, chemotaxonomic characteristics, and reproductive compatibility of a large number of lines of *V. radiata-mungo* complex collected from National Bureau of Plant Genetic Resources, India, and Asian Vegetable Research and Development Center in Taiwan. As a result, the differentiation of lines within the wild forms was confirmed, and their phylogenetic relationship was made clear.

Taxonomic groups of *V. radiata-mungo* complex

A total of 40 lines of mungbean, 32 lines of black gram, and 8 lines of wild form were examined for 7 characters such as morphology of pods and seeds, etc., which are known as the morphological indices for taxonomic classification. By using the principal components analysis, the quantitative characters were collectively assessed.[15] As shown in Fig. 1, in which scores of the first and the second principal components for each line are plotted, lines of mungbean are clearly distinguished

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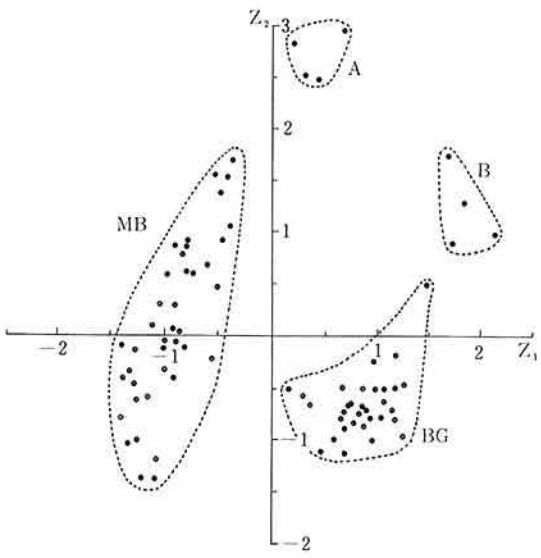

Fig. 1. Classification of *Vigna radiata-mungo* complex by principal components analysis (Miyazaki, 1982)

from those of black gram and the wild forms can be divided into two groups. The first principal component is mainly related to pod length and hilum thickness, while the second one mainly to seed size. Since these characters are known to be effective in distinguishing mungbean from black gram, and cultivated species from wild forms,[9] the two groups of the wild form lines were named A and B groups, and they were compared with each other for other several taxonomic characters. As a result, it was made clear that the A group has characteristics corresponding to mungbean, while the B group to black gram (Table 1).

In addition to such quantitative, morphological characters, qualitative differences were also examined. An example is chemical composition of anthocyanin contained in hypocotyls. It was found out to be useful as a tool for the classification of *V. radiata-mungo* complex.[8] Anthocyanin, a plant pigment, differs in kinds of anthocyanidin and in kinds and number of sugars attached to it, according to genus and species, and hence it has been regarded to be specific to genus and species.

Table 1. Variations of morphological characters in *Vigna radiata-mungo* complex

Characters	Mungbean	Wild form		Black gram
		A	B	
Hilum shape (length/thickness)	8~14	5~ 7	3~4	4~ 7
Pod shape (length/width)	15~27	11~14	8~9	8~10
Pod setting	Spreading	Spreading	Sub-erect	Sub-erect

It was found out that red-colored hypocotyls of mungbean and black gram contain two kinds of anthocyanin, respectively, one of which is common to both species, while the other is specific to each species. The common anthocyanin was delphinidin 3-glucoside. The one specific to mungbean was delphinidin 3-*p*-coumaroylglucoside, while the one specific to black gram was cyanidin 3-glucoside.[5]

The A group of wild form, resembling mungbean in morphological characteristics, showed delphinidin 3-*p*-coumaroylglucoside, which is specific to mungbean, in hypocotyls of all lines tested. On the contrary, the B group, resembling black gram in its morphology, produced cyanidin 3-glucoside, which is specific to black gram, without exception (Table 2). In the case of *V. radiata-mungo* complex, whose taxonomic classification is confused due to morphological similarity, the difference in anthocyanin composition seems to offer an important evidence to the study of affinity and differentiation of lines.

Table 2. Anthocyanin components in the hypocotyls of *Vigna radiata-mungo* complex

Anthocyanins	Mungbean	Wild form		Black gram
		A	B	
Delphinidin 3-glucoside	+	+	+	+
Delphinidin 3-*p*-coumaroyl-glucoside	+	+	−	−
Cyanidin 3-glucoside	−	−	+	+

+ present, − absent

Differences in some chemical components and physiological reactions in plants were also observed among the groups of *V. radiata-mungo* complex. TTC (triphenyl tetrazolium chloride) reduction of cotyledons, difference in esterase isozyme, etc. showed the same result as that of anthocyanin.[8,9]

Those results indicate, similarly to the grouping by morphological characteristics, that the wild forms are composed of two groups, corresponding to mungbean and black gram. Accordingly, the former is called mungbean-type wild form, and the latter black gram-type wild form.

Reproductive isolation in *Vigna radiata-mungo* complex

In general, reproductive isolation, such as cross-incompatibility, hybrid inviability, hybrid weakness, hybrid sterility, etc. offers extremely important information regarding species differentiation and phylogenetic relationship. In order to make clear the internal structure of the four taxonomic groups and the relation among them, a crossing experiment was carried out by using 7 lines of mungbean, 4 lines each of mungbean-type and black gram-type wild forms, and 3 lines of black gram, totaling 18 lines. The results are compiled in Fig. 2.[9]

All the crossings within each group resulted in normal seeds, and no hybrid weakness was recognized in the growth of F_1 plants. However, within the group of mungbean-type wild form, several combinations showed a marked lowering of F_1 pollen fertility. It indicates that the group involves relatively wide genetic variations.

Of the crossings among the four groups, those between mungbean and mungbean-type wild forms, and between black gram and black gram-type wild forms resulted in normal hybrid seeds, without hybrid weakness in F_1 plants, although some lines showed a reduced F_1 pollen fertility. This result coincides with that of crossings within each group. Thus, it was confirmed that mungbean and mungbeant-type wild forms, and black gram and black gram-type wild forms are extremely alike, respectively in taxonomic relation.

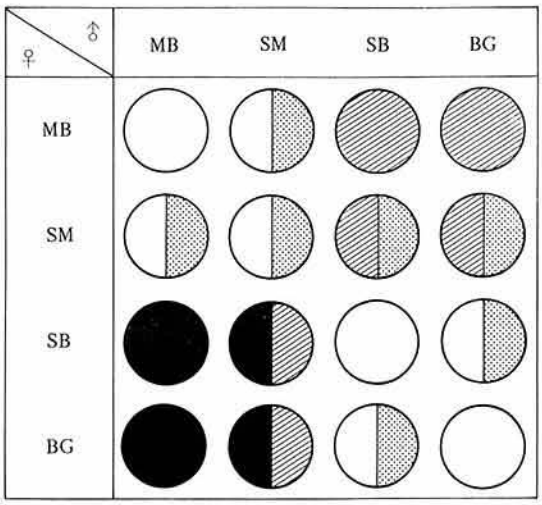

Fig. 2. Reproductive isolation in *Vigna radiata-mungo* complex (Miyazaki, 1982)

However, in combinations other than the above two, hybrid inviability, hybrid weakness, hybrid sterility, etc. were apparently observed as shown below.

First, in the crossing between mungbean and black gram, the F_1 seeds which have germination ability were obtained only when mungbean was used as the female parent, as was already reported by others.[1,4] However, all these seeds were morphologically quite abnormal: some seeds showed broken seedcoat from which cotyledon was protruding, and others were not fully ripened ones (Plate 1 and 2). This result suggests that the two species are in a fairly distant relation.

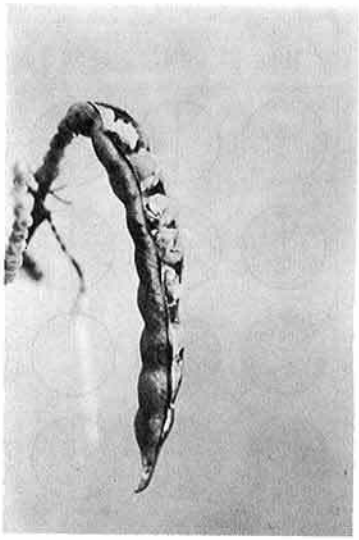

Plate 1. Abnormal pod formation observed in the cross between mungbean and black gram (Miyazaki, 1982)

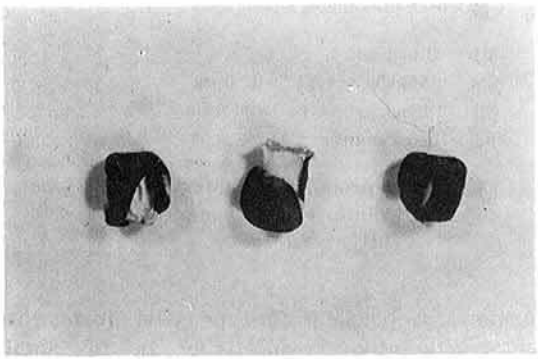

Plate 2. Morphological abnormality of F_1 seeds between mungbean and black gram (Miyazaki, 1982)

Second, in the crossings between the mungbean-type wild form and either black gram or black gram-type wild form, interesting phenomena, not observed in the above-mentioned crossing between mungbean and black gram were recognized. In the case when mungbean type wild form was used as the female parent, the remarkable hybrid weakness generally occurred, but Plu-270a, one of the mungbean-type wild form used, showed a different result. In the combinations with any lines of black gram and black gram-type wild form, used as male parents, Plu-270a produced F_1 plants which grow quite vigorously. In another case when the mungbean-type wild form was used as the male parent, only empty seeds without germination ability were produced in general. But, TC 1965, a wild form used, was an exception. When any lines of black gram or black gram-type wild form were used as the female parent, they produced F_1 seeds with germination ability, and the seeds were as large as those produced by selfing. Although the F_1 plants stop growing at the seedling stage and soon die, it is noteworthy that the hybrid was obtained by the reciprocal crossing, which was never successful between cultivated species.

From these results of the crossing experiments, it was confirmed that the wild forms are composed of two genetically different groups, one is similar to mungbean and the other to black gram. In addition, it was made clear that the mungbean-type wild forms maintain a wide variation regarding reproductive separation even at present. This fact suggests that the ancestral species of *V. radiata-mungo* complex was near to the present mungbean-type wild form, and, that in the ancestral species, differentiation of black gram type, which later became black gram by cultivation, had occurred.

Classification of *V. radiata-mungo* complex

As *V. radiata* and *V. mungo* have respective characteristics, clearly different in morphology and chemical composition of plant, and, furthermore, hybrid inviability and hybrid weakness occur generally in the crossing between them, it seems reasonable to identify them as two different species. On the other hand, mungbean-type wild form and black gram-type wild form can be regarded as the same species as *V. radiata* and *V. mungo*, respectively, judging from degree of reproductive isolation expressed among the 4 taxonomic groups of *V. radiata-mungo* complex. Since there is not necessarily a consistent view at present regarding the taxonomic orientation

of a related wild form, which belongs to the same species as the cultivated species, the present authors refer the wild form to a variety.[14]

Namely, as the mungbean-type wild form is the same as what was described as var. *sublobata* (personal communication of Ohashi), it may reasonably be termed *V. radiata* (L.) Wilczek var. *sublobata* (Roxb.) Verdec. On the other hand, black gram-type wild form may be termed *V. mungo* (L.) Hepper var. *silvestris* Lukoki, Maréchal & Otoul, on the basis of the recent theory by Lukoki et al.[7]

As mentioned above, mungbean-type wild form is extremely rich in genetic variation, and hence some lines which do not meet the above classification might be discovered with the progress of wild form collection in future. At the present moment, however, much of the contradictory information so far obtained seem to be settled down by classifying mungbean-type wild form as a variety of *V. radiata*, and black gram-type wild form as a variety of *V. mungo*.

The above result also suggests that the collection of wild forms, particularly of mungbean-type, is important to clarify the differentiation of *V. radiata-mungo* complex.

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(Received for publication, April 1, 1983)