Development of physical color reference for Indonesia paddy collection

N Hidayatun[1]*, M Sabran[1], A Ramadhani[1] and N A Widaningsih[2]

[1] Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture, Jalan Tentara Pelajar No. 3A, Bogor 16111, West Java, Indonesia
[2] Indonesia Center for Agricultural Permit and Plant Variety Protection, Jalan Harsono R.M. No. 3, Ragunan, Pasar Minggu, South Jakarta, Jakarta 12550, Indonesia

*E-mail: nurulhi23@yahoo.com

Abstract. One of the objectives of genebank management is to maintain the genetic identity of its collection. In order to maintain the genetic identity, it is important to check new seed against a reference collection. A simple morphological character for determining the reference collection is the color of lemma and palea. Lemma and palea is a pair of bract-like organ in the floret and have similar pattern of pigmentation and therefore can be treated together. The color of lemma and palea determine the seed color. To develop reference collection for grain color characterization of rice germplasm in the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) Genebank, 1,000 sample of seeds produced from different year of regeneration are characterized based on the lemma and color categories of the IRRI guidelines for the characterization and evaluation system. Samples of each category were then arranged and photographed. From 11 categories of lemma and palea color described in the guidelines, there are six color categories available from genebank rice collection, whereas five color categories were not found. The six available color categories were straw, golden, brown spot in the straw background, brown line in the straw background, brown, orange-brown and black. Five color categories were not found in the collections i.e. red-purple, purple spots on straw, purple line on straw, purple and white. This material reference could be used for characterization of Indonesian rice. The seeds of the absent categories might need to be aquisitated to the genebank to complete the reference collection.

Keywords: material references, grain color, rice characterization.

1. Introduction

Rice is staple food for Indonesian and most people in South East Asian countries. Indonesia possess high genetic diversity of rice germplasm [1–3]. To ensure the sustainability of rice production in the changing environment, in particular climate changes, Indonesia needs to conserve its rice germplasm *ex situ* as well as on-farm. The National Genebank constructed in 2015 hosted by the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), designated as the main genebank to conserve the core collection of rice in Indonesia; although it may be functioned as *ex situ* conservation for other crop as well [4]. The genebank collects landraces from all regions in Indonesia, as well as improved cultivar and wild relatives. As a new genebank, it has several shorthcoming, e.g. no protocols for sorting the new accession, regeneration and transfer of the materials, duplication of materials, in addition of lack of qualified technician and funding. The number of collections is also limited compared with the capacity of the genebank and the diversity of crops including rice in Indonesia. One important step to overcome those shorthcoming is by improving the genebank management.

The main objectives of genebank management are the preservation of genetic identity and integrity of the germplasm as well as, maintenance of the seed, viability and the promotion of access [5]. Accession maintained in genebank should be remained genetically as similar to the original collected materials as possible. The usual method to check the genetic identity is by checking the morphological traits against descriptor list. Changes in genetic integrity may be caused by genetic drift, unintentional selection and pollen contamination during the regeneration of collection; in addition to mislabelling and seed contamination during the germplasm handling. The greatest change in genetic integrity is the transfer of materials between genebanks. Even a duplicate of a collection might changes its integrity when under the management of different genebank [6]. It is, therefore, important to improve both the handling of accessions within genebank and the protocol for transfer of accessions between genebanks.

Although molecular tools are now available to precisely check and monitor the genetic identity and integrity at the genotype level, morphological characterization can still be used. Characterization facilitates the effort for discriminating phenotypes and allows simple grouping of the accessions [7] Moreover, the characters can be used to facilitate utilization of collections and to detect misidentification and possible errors during genebank operations. Characterization also provides a better insight into the composition of the collection and the coverage of genetic diversity. There are internationally agreed descriptors for characterization, however, characterization is somewhat labour-intensive. Data recording needs to be carried out by trained staff using calibrated and standardized measuring formats, as indicated in the internationally agreed crop descriptor [5].

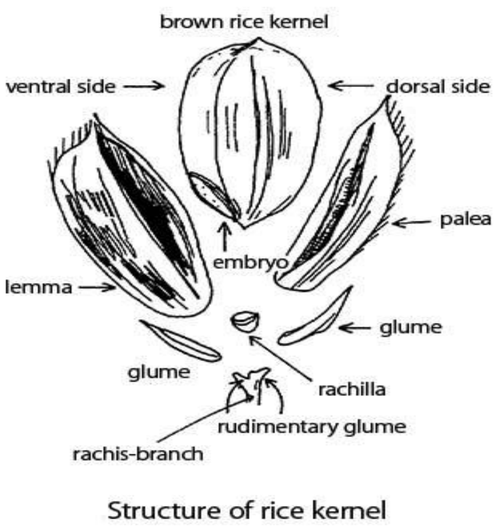

Figure 1. Structure of rice kernel.

Reference collections play an essential role in true-to-type identification [5]. This sample reference can facilitate the identification and characterization process. In early sorting stages of new seeds for the collection, it is important to have reference collection for certain characters that are easy and simple to observe. The lemma and palea color are the simplest morphological character to group rice seed, and therefore, can be used to categorize rice accession at the premilinary sorting of the new accession. The color is also useful in the eradication of wild type in the cultivated rice, in particular in the farming region where wild type of rice can only be differentiated among purple rice plant or rice plants having some pigmented parts like Madya Pradesh, India. Patil and Sahu [8] suggest the development of varieties with pigmentation to overcome this problem. Lemma and palea are a pair of bract-like organ in the floret (Figure 1) [9]. There is a wide diversity in the distribution, intensity and location of pigment in the lemma and palea. Both lemma and palea show a similar pattern of pigmentation, and therefore, are treated together [10].

The objective of the study is to develop material references for color identification on Indonesia rice germplasm. The availability of physical reference will help curator and technician to characterize and identify rice accession. This will facilitate the characterization program, particularly on the character of rice color.

2. Materials and methods

The color reference was developed based on the rice seed samples of ICABIOGRAD Genebank. A total of 1,000 samples of seed from different years of generation were sampled and characterized. The seed samples were grouped based on the color of lemma and palea in accordance with the IRRI Rice Descriptor [11]. The categories and the availability of each category in the ICABIOGRAD Genebank collection are presented in Table 1. Each seed sample in each category is assigned as sample reference. The samples were photographed and stored in an appropriate pack according to the designation. The photograph was arranged as a paper-printed color reference.

3. Results and discussion

According to the Guidelines for Characterization and Evaluation of Rice [12], there are 35 rice morphological characters, in addition to 12 agronomical characters and 14 characters associated with grain quality. In addition to those characters, there are characters related to symptoms to diseases (23 characters), plant broken due to rodent and birds (2 characters), plant broken caused by insects (11 characters), physical-chemist stresses (4 characters) and characters related to tolerance to deep water such as elongation, submergence tolerance and survival. There are also characters for evaluation of hybrid rice and its progenitors.

There are at least nine characters that are usually used to describe rice seed, i.e. lemma and palea color, the existence of hair in the lemma and palea, the color and the length of sterile lemma, the color of epiculus and the existence of hair on the epiculus and the color and the length of the hair in the epiculus. In the rice seed structure, lemma and palea are the two parts attributing the grain color. Lemma and palea are perianth organs that surround the inner floral organs. Thus, these two organs determine the whole unhusked-grain color [9].

Table 1. Categories of rice seed color and the availability of sample representing each category.

Category	Description	Availability on the collection
0	Straw	v
1	Gold and gold furrows	v
2	Brown spots on straw	v
3	Brown furrows on straw	v
4	Brown (tawny)	v
5	Reddish to light purple	-
6	Purple spots on straw	-
7	Purple furrows on straw	-
8	Purple	-
9	Black	v
10	White	-

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As indicated in Table 1, there are 11 categories of the color of lemma and palea, and six categories are available in the ICABIOGRAD Genebank rice collection (Figure 2). The straw color category is the most common category found in the seed collections. The dominance of this color was also found in a study [8] on rice cultivation in Chhattisgarh, India.

Figure 2. Six color categories found in ICABIOGRAD-IAARD sample collection.

No sample found among the seed stock represented the purple group and white color categories. The purple group color categories consisted of four categories, i.e. reddish to light purple, purple spots on straw, purple furrows on straw and purple. The unavailability of those samples is probably due to the lack of genetic materials representing the color variation, or, merely due to the maturity level of the seed materials used in the study.

The purple pigment is controlled by the *Hep* gene [10]. Spikelet color is somewhat different in the flowering to ripening stage. However, no references found regarding the effect of storage duration on the color change of lemma-palea. At ripening, the color of lemma and palea changes into a quite different one [10]. At flowering, anthocyanin distribution in lemma and palea could be categorized as purple tips, purple spread and full purple. The ripening color of lemma-palea acted independently of the color of the early stage. The same color of the lemma-palea in the growing stage might ripen into different color at the ripening stage. Conversely different color of the young probably ripens to the same color at maturity (Figure 3).

Bioversity International [13] determine lemma-palea color differently in early and late observation. There are 15 color categories for early observation, whereas only 11 categories for the late observation. The pre-ripening stage, considered as early observation, is supposed to be conducted after anthesis to hard dough stage. The Bioversity descriptor is subjected to both cultivated rice and wild relatives. Considering that the rice collections consisted of landraces and local varieties, the availability of this descriptor in the collection might open the possibility of expanding the category of this lemma-palea color.

For the *Oryza sativa*, the lemma-palea color is supposed to be characterized at the maturity stage or when the terminal spikelets are ripened [11]. Samples used for color determination in this study were stock samples that were ripen harvested materials and have been stored for years. This level of maturity should provide the final and stable lemma-palea color. Thus, the unavailability of some color categories could be assumed as the lack of materials in the genebank collection. This should be considered for the next acquisition program. Exploring materials with the particular lemma-palea color is needed in order to fill the gap of the genetic variation.

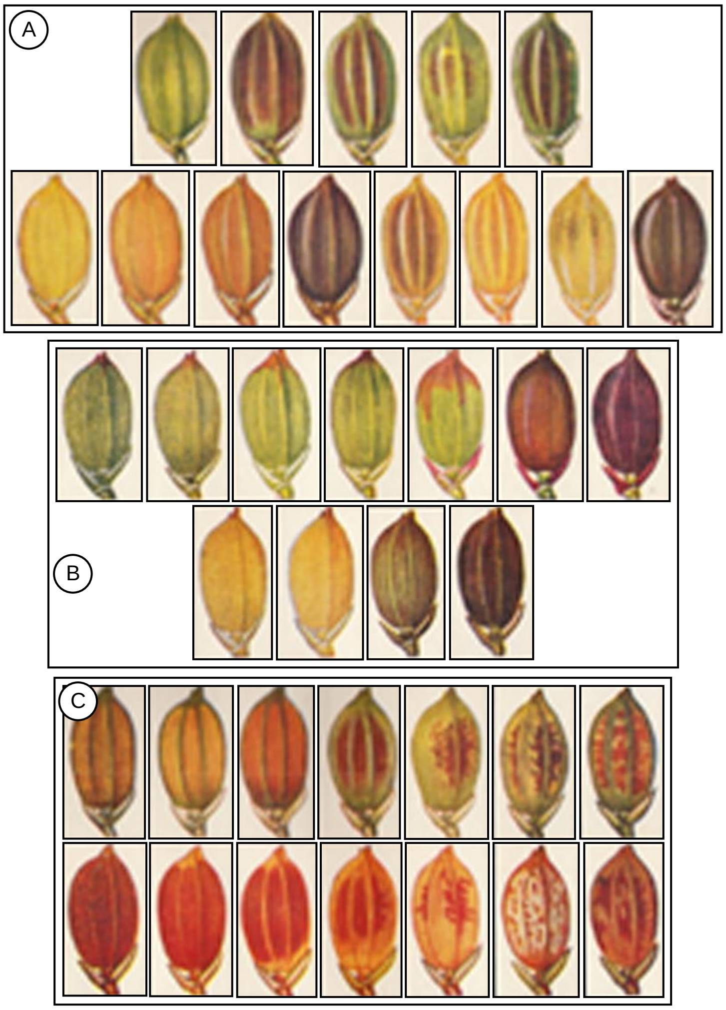

Figure 3. The color change of lemma-palea at flowering and maturity stage [10]. (A) Some color category of flowering changed to broader range of color category at maturity. (B) Some color category of flowering changed to narrower range of color category at maturity. (C) Color category at maturity.

4. Conclusions

There are six color categories found in the stock materials of rice collection in ICABIOGRAD Genebank rice collection. These stock materials can be used as a color reference for characterization of lemma-palea color. Five color categories are not found in the collection. The information about the lack of the five color categories is also useful for targetting the next acquisition plan. The genebank needs to acquire seed samples representing these five colors in order to establish a complete reference. Another consideration is to formulate the need for early-observation of lemma-palea in the pre-ripening stage by using the descriptor by Bioversity international.

In summary, we present three core collections that have their value for the landscape's genetic resources management, research and breeding. We believe that the methodologies implemented in this work may be successfully extrapolated to other rice landraces and cultivars, with similar results when selecting a core collection from a highly related original collection.

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5. Authors contribution

NH and MS are the main authors of the manuscript contributed equally in the design and interpreting the observation on the samples. AR contributed in observation and in preparing the sample materials. NAW contributed in adding several paragraphs and commented on the manuscript.

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