January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Rejuvenation and morphological characterization of local rice from the province of Yogyakarta

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Setyorini Widyayanti, Sutarno, Endang Wisnu Wiranti, et al.

Rejuvenation and Morphological Characterization of Local Rice from the Province of Yogyakarta

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Abstract. Rejuvenation is one of the efforts in *ex situ* conservation to improve the viability and availability of seed collection. Morphological characterization is important in plant breeding. It can be used to classify genotypes to find out relationship between genotype which is used as reference material in selection. The Province of Yogyakarta has various genetic resources of local rice obtained through several years of exploration, but some have not been known for its potential superiority. Some of these local rice collections are currently limited in seed number, morphological, and other important characteristics data are still lacking. Therefore, rejuvenation and characterization are needed for providing breeding materials. This research aimed to rejuvenate and to evaluate the morphological characters of 15 Yogyakarta local rice. Rejuvenation and characterization of the varieties in vegetative and generative growth stages were carried out in 2019 at a screen house in Banyakan, Bantul Regency. The result showed that rejuvenation of 15 local rice produced 13 cultivars which yielded >100 g of seeds per cultivar. Based on quantitative character, 15 local rice could be grouped into three main groups. The first group consisted of 12 local rice, the second group consisted of 2 local rice, and the third group consisted of 1 local rice. The main determinant characters were days of maturity, plant height, number of grains per panicle, and grain weight. Based on the qualitative character, 15 local rice could be grouped into two groups. First group consisted of 12 local rice and the second group consisted of 3 local rice. The most determinant characters were grain color and flag-leaf attitude. Grouping local rice based on its morphological characters can be used as a reference in selection. In this case, the effectiveness of selection can be easily understood by using color clustergram visualization.

INTRODUCTION

The Province of Yogyakarta (Daerah Istimewa Yogyakarta) stretches quite broadly from south coastal area, lowlands area, upland area, karst material, mangrove area to Mount Merapi [1]. With various landscapes, it can be expected that this province has high plant genetic resources variation. Local rice is cultivars which are planted for generations and are adapted to specific land and climate condition. Exploration has been carried out to collect Yogyakarta local rice diversity since 2014 until now. The local rice collection needs to be maintained and appropriately managed to avoid extinction.

Ex situ conservation is an effort to manage genetic resources outside their original habitat [2], which can be carried out through rejuvenation. Rejuvenation is also intended to improve the viability of seeds from exploration which have deteriorated due to the long-term storage process. Rejuvenation is carried out periodically every 2–3 years. This was specially performed on seeds with decreased germination by up to 80%, so new seeds with high viability can be obtained [3, 4].

Characterization is an activity to record and document important characters that can be inherited, specific, and can be used to distinguish within and among families in a plant species [5, 6]. Characterization can be done through several approaches of identification. The easiest one is by morphological characterization [7] that is believed to be more accurate for identifying individual phenotype performance and changes related to the growth environment [6, 7].

Yogyakarta has various local rice collection through several years of exploration. It has reached 180 local rice accessions, consisting of 76 accessions of white rice, 26 accessions of red rice, 41 accessions of black rice, and 37

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accessions of sticky rice till 2018 [8]. The characters of some Yogyakarta local rice are not yet known. The morphological characters are needed to determine the potential superiority of rice that could be beneficial for screening parents for crossbreeding programs. In addition, it can be used in evaluating biotic resistance and abiotic tolerance. Therefore, the purpose of this study was to rejuvenate and to evaluate the morphological characters of local rice cultivars from The Province of Yogyakarta.

MATERIALS AND METHODS

This study was carried out at screen house of Yogyakarta Assessment Institute for Agricultural Technology which is located in Banyakan, Bantul Regency from April to October 2019. The genetic material consisted of 15 accessions of local rice from the Province of Yogyakarta (Table 1). Each accession was planted in a 15-l plastic pot containing a mixture of soil and organic fertilizer in the ratio of 1:2, with three replications each (1 replication = 1 pot). Each pot is planted with 2 seeds. Plants were fertilized with 250 kg/ha urea, 100 kg/ha TSP, and 100 kg/ha KCl. Plant maintenance included irrigation and control of pests and diseases as necessary.

Qualitative and quantitative morphological characterization was carried out by referring to wild type and cultivated rice guideline descriptions issued by international bioversity agencies [9]. Qualitative morphological characters, which cannot be measured in units [10], were scored according to the scaling system provided in the guideline. These characters included auricle color, flag-leaf attitude, ligule color, leaf upper surface, ligule shape, leaf blade color, grain color, pericarp color, and collar color. Quantitative morphological characters can be measured by tools [10]. Quantitative characters observed included plant height (cm), days of maturity (days after seedling), leaf length (cm), leaf width (cm), panicle branch length (cm), grain length (cm), grain width (cm), weight of 1,000 grains (g), number of grain per panicle, number of grain per panicle in percentage (%), number of empty grain per panicle, and grain weight (g).

Mean and standard deviation were calculated by using Microsoft Excel software. Cluster analyses were performed by using the R-package d4 Heatmap software.

TABLE 1. List of local rice materials from the Province of Yogyakarta evaluated in this study.

Code	Registration number	Name of accession
YK 1	BPTPYK00187	Rojolele Marjan
YK 2	BPTPYK00163	Biosa
YK 3	BPTPYK00047	Menor
YK 4	BPTPYK00133	Pandanwangi Muharjo
YK 5	BPTPYK00153	Andel Lele
YK 6	BPTPYK00157	Cempo Malog
YK 7	BPTPYK00177	Mentik Grompol
YK 8	BPTPYK00001	Batang Lembang
YK 9	BPTPYK00077	Pangestu
YK 10	BPTPYK00189	Menor A1
YK 11	BPTPYK00010	Dewi Mayangan Handayani
YK 12	BPTPYK00136	Similikti
YK 13	BPTPYK00188	Raja Limba Bleder
YK 14	BPTPYK00190	Cempo Welut
YK 15	BPTPYK00123	Rening

RESULTS AND DISCUSSIONS

Rejuvenation

The local rice accessions generally showed good growth performance in both vegetative and generative phases during rejuvenation. All accessions were able to grow and develop well indicating that the seed storage process has

functioned well. Rejuvenation generally produced ≥50 g seeds [11]. Indeed, ≥50 g seeds per accession were generally produced (Table 2). Thirteen accessions had seed weight ≥100 g, 1 accession (YK 1) produced seeds with weight >50 g, while one accession (YK 13) produced seeds with weight of <50 g. Generally, this rice collection is classified as local rice which does not fall off easily. The level of grain maturity is perfect, indicated by the low percentage of empty grain. The number of grain per panicle, panicle branches, and days of maturity affect the number of regenerated seeds produced [11].

Quantitative Morphological Characterization

Characterization of Yogyakarta local rice is divided into quantitative characters in accordance with the growth phase. Plant height varied from 100.67 to 187.33 cm (Table 2). According to the rice characterization guideline, lowland rice plant height is classified into short (<100 cm), medium (110–130 cm), and high (>130 cm) [9]. Accessions with short plant height were YK 8, YK 9, YK 12, and YK 15; those with medium plant height were YK 2, YK 4, YK 7, YK 13, and YK 14; whereas those with high plant height were YK 1, YK 3, YK 5, YK 6, YK 10, and YK 11.

The average days of maturity reached 155 days after seeding (DAS). The criteria for days of maturity are divided into deep maturity (151 DAS), medium maturity (125–150 DAS), early maturity (105–124 DAS), very early maturity (90–104 DAS), and ultra-early maturity (<90 DAS) [12]. Based on these criteria, only YK 11 was classified as having medium maturity (127 DAS), whereas the remaining accessions had deep maturity.

TABLE 2. Quantitative characters of local rice from the Province of Yogyakarta.

Code	Registration number	PH (cm)	DM (DAS)	LL (cm)	LW (cm)	PB (cm)	GL (cm)	GW (cm)	W1000 (g)	NFG	NEG	PNFG (%)	GWs (g)
YK1	BPTPYK00187	187.33	132	65.87	1.80	27.40	0.82	0.29	29.50	178	37	82.79	63.74
YK2	BPTPYK00163	115.33	134	30.36	1.10	23.47	0.92	0.21	26.40	74	6	92.50	138.01
YK3	BPTPYK00047	131.67	152	36.50	1.67	26.20	0.83	0.30	23.80	108	6	94.74	156.98
YK4	BPTPYK00133	124.33	152	39.40	1.70	27.07	0.88	0.28	30.60	120	12	90.91	173.91
YK5	BPTPYK00153	146.33	150	32.29	1.40	27.33	0.77	0.26	26.60	145	7	95.39	179.27
YK6	BPTPYK00157	169.67	150	37.70	1.50	24.13	0.73	0.29	25.90	192	5	97.46	179.61
YK7	BPTPYK00177	116.33	167	39.17	1.67	24.92	0.74	0.31	28.50	208	7	96.74	208.59
YK8	BPTPYK00001	106.33	138	36.10	1.47	21.17	0.87	0.22	23.60	133	8	94.33	137.93
YK9	BPTPYK00077	101.00	161	53.60	1.30	21.83	0.88	0.23	24.40	152	7	95.60	125.61
YK10	BPTPYK00189	158.00	152	59.00	1.40	35.93	0.93	0.27	31.90	200	53	79.05	186.69
YK11	BPTPYK00010	178.67	127	61.33	1.83	33.23	0.90	0.28	28.20	177	13	93.16	104.22
YK12	BPTPYK00136	101.17	160	46.57	1.53	25.33	0.86	0.29	32.90	123	14	89.78	174.73
YK13	BPTPYK00188	126.63	260	69.93	1.70	21.15	0.85	0.26	12.40	22	95	18.80	20.19
YK14	BPTPYK00190	124.33	160	37.50	1.33	23.47	0.81	0.22	18.80	117	5	95.90	112.22
YK15	BPTPYK00123	100.67	132	33.07	1.58	25.80	0.82	0.27	25.40	185	17	91.58	151.48
Average		**132.52**	**155.13**	**45.23**	**1.53**	**25.90**	**0.84**	**0.27**	**25.93**	**144.71**	**19.47**	**87.25**	**140.88**
Standard deviation		**28.00**	**30.45**	**12.75**	**0.20**	**3.98**	**0.06**	**0.03**	**5.02**	**50.12**	**24.01**	**0.19**	**48.44**

PH = plant height, DM = days of maturity, LL = leaf length, LW = leaf width, PB = panicle branches length, GL = grain length, GW = grain width, W1000 = weight of 1,000 grains, NFG = number of filled grains per panicle, NEG = number of empty grains per panicle, PNFG = percentage of number of filled grains per panicle, GWs = grain weights, DAS = days after seeding.

Leaf length is divided into five categories, namely very short (<21 cm), short (21–40 cm), medium (41–60 cm), long (61–80 cm), and very long (>80 cm) [9]. The local rice has an average leaf length of 45.23 cm and leaf width of 1.53 cm (Table 2). Nine accessions had short leaf length; YK 9, YK 10, and YK 12 had medium leaf length, meanwhile the remaining accessions (YK 1, YK 11, and YK 13) could be classified to have a long leaf. Panicle branches length is one of the characteristics which can affect yield, so it can be used as an indicator of grain

production capacity [13]. The average panicle branches length of the local rice was 25.90 cm. The grain length and grain width were fairly uniform. Grain length ranged from 0.78 to 0.90 cm, whereas grain width ranged from 0.24 to 0.30 cm. Saryoko *et al*. [14] stated that most Indonesian people prefer long and uniform rice sizes. All local rice studied has the potentials to be used as the parental selection in crossbreeding program.

In addition to panicle branches length, weight of 1,000 grains is another morphological character which can affect rice yield [13]. The average weight of 1,000 grains was 25.93 g. FAO [15] classifies the weight of 1,000 grains into very heavy (>28 g), heavy (22–28 g), and light (<22 g). Based on this classification, there were 6 accessions that fall into the very heavy category, namely YK 1, YK 4, YK 7, YK 10, YK 11, and YK 12. A total of 7 accessions were classified into heavy grain criteria, namely YK 2, YK 3, YK 5, YK 6, YK 8, YK 9, and YK 15, whereas 2 accessions (YK 13 and YK 14) were classified into light grain criteria.

A number of grains per panicle is an indication of the potential yield of rice accession. Dewi *et al*. [16] stated that high yielding rice has a range of 150–250 grains per panicle or 85–95% of the total number of grains per panicle. Rejuvenation indicated that 13 rice accessions were potential to produce the high number of grains. The number of grains per panicle was >89%, except YK 1, YK 10, and YK 13 that produced 82.79%, 79.05%, and 18.80% grains number per panicle, respectively. These yield potentials were reflected in grain weight per hill.

Qualitative Morphological Characterization

TABLE 3. Qualitative characters of local rice from the Province of Yogyakarta.

Code	Registration number	FL	LUP	AC	LS	LC	CC	LB	GC	PC
YK1	BPTPYK00187	Semi-erect	Hairy	Whitish	2 cleft	Whitish	Light green	Dark green	S	White
YK2	BPTPYK00163	Erect	None	Whitish	2-cleft	Whitish	Light green	Dark green	S	White
YK3	BPTPYK00047	Erect	None	Whitish	2-cleft	Whitish	Light green	Light green	S	White
YK4	BPTPYK00133	Semi-erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Light green	S	White
YK5	BPTPYK00153	Semi-erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Dark green	S	White
YK6	BPTPYK00157	Semi-erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Dark green	YC	White
YK7	BPTPYK00177	Semi-erect	None	Whitish	2 cleft	Whitish	Light green	Light green	S	White
YK8	BPTPYK00001	Erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Light green	S	White
YK9	BPTPYK00077	Erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Green	S	White
YK10	BPTPYK00189	Semi-erect	Semi-hairy	Whitish	2-cleft	Whitish	Light green	Green	S	White
YK11	BPTPYK00010	Erect	None	Whitish	2-cleft	Whitish	Light green	Light green	S	White
YK12	BPTPYK00136	Erect	None	Whitish	2-cleft	Whitish	Light green	Green	B	White
YK13	BPTPYK00188	Semi-erect	Hairy	Whitish	Acute-acuminate	Whitish	Light green	Green	B	White
YK14	BPTPYK00190	Erect	Hairy	Whitish	2-cleft	Whitish	Light green	Light green	B	White
YK15	BPTPYK00123	Semi-erect	None	Whitish	2-cleft	Whitish	Light green	Light green	YC	White

FL = flag-leaf attitude, LUP = leaf upper surface, AC = auricle color, LS = ligule shape, LC = ligule color, CC = collar color, LB = leaf blade color, GC = grain color, PC = pericarp color, S = yellow straw, YC = yellow straw with brown stripe, B = brown.

To complement the quantitative characters, the qualitative characters of these local rice observed included auricle color, flag-leaf attitude, ligule color, leaf upper surface, ligule shape, leaf blade color, grain color, pericarp color, and collar color. Eight accessions had erect flag leaf, whereas 7 accessions had semi-erect flag leaf (± 45° angle). The leaf upper surface varied, from none hairy, semi-hairy, and hairy. Auricle and ligule color were uniform, namely whitish (colorless).

The majority of ligule shapes were 2-cleft, but one accession (YK 13) had acute-acuminate ligule. All accessions had light green collar color. Leaf blade color varied from light green, green to dark green. The leaf morphology of local rice is one of main selection in plant breeding program especially hybrid rice variety [17]. According to Rohaeni and Yuliani [17], leaf upper surface, leaf blade color, and collar color has correlation with bacterial leaf blight (BLB) disease resistance. Thus, this Yogyakarta local rice diversity can be a preference on plant breeding program based on leaf morphological character against BLB disease resistance. Grain color was very diverse [9]. The grain color of 3 accessions (YK 12, YK 13, and YK 14) was brown, 2 accessions (YK 6 and YK 15) yellow straw with brown stripe, and the grain color of the remaining 10 accessions was yellow straw. Fifteen Yogyakarta local rice color preference is the same as local rice color preference, yellow straw [18, 19]. Generally, it can be concluded that local rice grain color is yellow straw and all accessions displayed white pericarp (Table 3).

Clustergram Analysis

Fig. 1 depicted the results of clustergram analysis. Rand column in the clustegram represent genotype and quantitative morphological characters, respectively, whereas red and green color represent the decreasing and increasing treatment effect, respectively. Based on the similarity of reciprocal patterns between morphological characters and genotypes, there are four groups of quantitative characters: group 1 include grain width (WG), grain length (GL), leaf width (LW), and number of empty grains per panicle (NEG); group 2 consists of leaf length (LL), panicle branches-length (PB), and weight of 1,000 grains (W1000); group 3 consists of grain weight (GW) and number of grains per panicle (NFG); group 4 consists of plant height (PH) and days of maturity (DM). These groupings can indicate the efficiency of the selection process in crossbreeding [13].

Three large groups of genotypes are observed in the clustergram. Group 1 consists of 12 genotypes which are further divided into 2 subgroups, each consists of 2 to 3 sub-subgroups. This clustergram describes the average genotype value, which can be seen in the distribution of colors for each character. Subgroup 1 consists of 7 genotypes which is predominated by days of maturity (DM) character compared to subgroup 2 which include 5 genotypes, but have lower characteristics of grain weight (GW), number of grains per panicle (NFG), and plant height (PH) than subgroup 2.

Group 2 consist of 2 genotypes, namely YK11 and YK1. The number of grains per panicle of this group is classified as large and the plant is tall as presented with the plant height (deep maturity) compared to the genotypes of group 1. Group 3, which contains only YK 13, is an outlier group. This genotype is characterized by very low grain weight and grains per panicle and very deep maturity. Based on morphological character, this accession is less potential as parental in crossbreeding. However, it is necessary to explore the potential or other advantages, such as biotic resistance and abiotic tolerance.

The difference between the genotype groups based on quantitative characters was visible as represented by different colors. The red color showed that leaf width (LW), grain width (GW), and grain length (GL) characters were the same for each genotype. The green color for grain weight (WG), number of grains per panicle (NFP), plant height (PH), and days of maturity (DM) characters indicate that these characters can be differentiating characters. The color in the genotype clustergram reflects a trend of recessive (red) into dominance (green) characters, therefore, it will be easier to understand the difference between genotypes [13]. For parental selection based on quantitative character, it is recommended that genotype 1, subgroup 2, i.e. number of grains per panicle and grain weight, can be chosen as dominant references.

Based on clustergram analysis of qualitative characters, the local rice was divided into 2 groups (Fig. 2). Group 1 included grain color (GC), pericarp color (PC), collar color (CC), and ligule color (LC), whereas group 2 consisted of flag-leaf attitude (FL), leaf blade color (LB), leaf upper surface (LUP), and ligule shape (LS). Group 1 comprised 2 subgroups, each of it had 2 to 4 sub-subgroups, while group 2 consisted of genotype YK 12, YK 13, and YK 14. Subgroup 1 was based on flag-leaf attitude. Accession YK 1, YK 4, YK 5, YK 6, YK 10, and YK 15 had a flag-leaf attitude with a lighter color intensity (green) than the remaining accessions (YK 2, YK 3, YK 8, YK 9, and YK 11). Group 2 which contained accession YK 12, YK 13, and YK 14, had more dominant grain color character than group 1. The color clustergram denoted that red color reflected recessive trend, while green color reflected

dominance trend character. According to Lee *et al*. [20], the higher color intensity of the genotype for a character, the higher genotype value of that character, indicating that visual color facilitates to understand the difference between genotype [13]. As consequence, selection of genotype for breeding materials could be directed from dominance character or recessive characters.

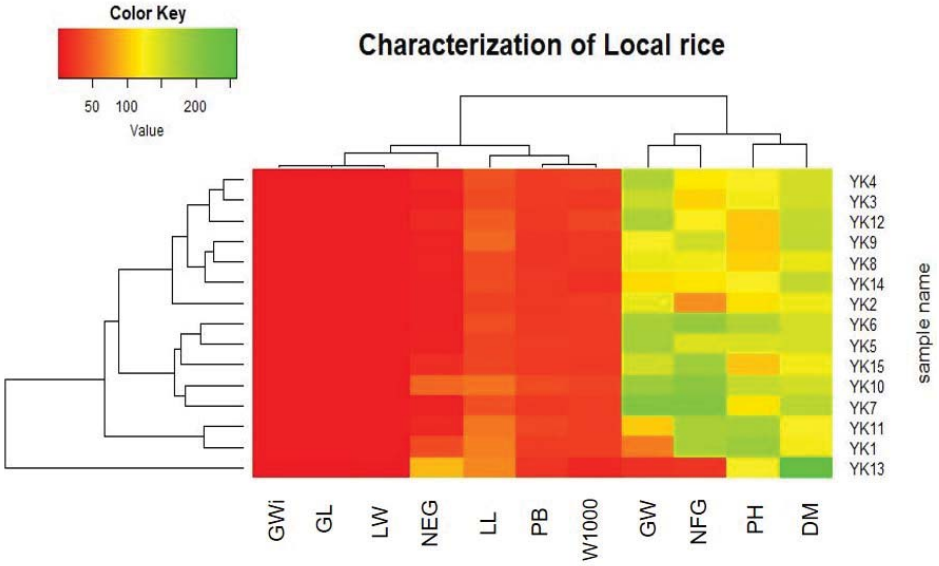

FIGURE 1. Clustergram analysis with the heatmap concept of 15 Yogyakarta local rice at Banyakan, Bantul Regency, the Province of Yogyakarta in 2019, based on quantitative character. DM = days of maturity, PH = plant height, LL = leaf length, LW = leaf width, PB = panicle branches length, GL = grain length, GWi = grain width, W1000 = weight of 1,000 grains, NFG = number of grains per panicle, NEG = number of empty grains per panicle, GW = grain weight.

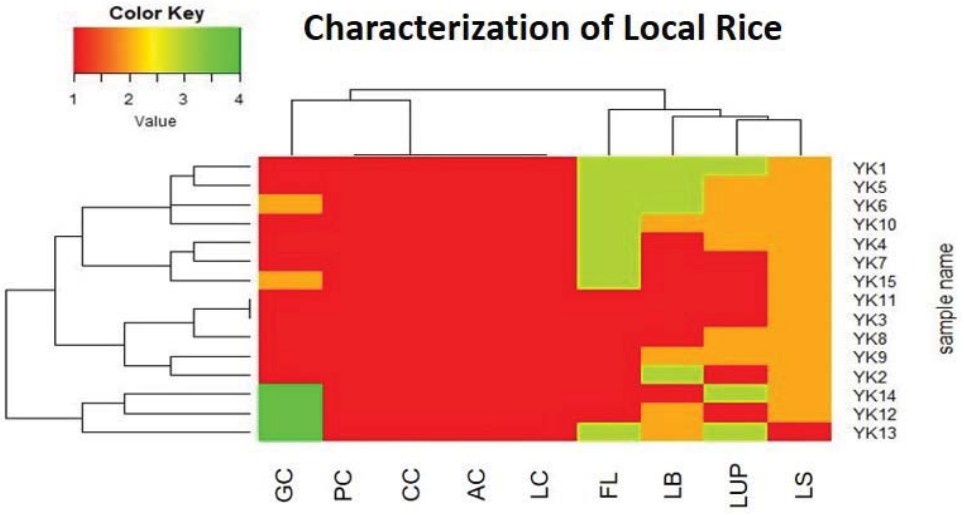

FIGURE 2. Clustergram analysis with the heatmap concept of 15 Yogyakarta local rice at Banyakan, Bantul Regency, the Province of Yogyakarta in 2019 based on qualitative character. GC = grain color, PC = pericarp color, CC = collar color, AC = auricle color, LC = ligule color, FL = flag-leaf attitude, LB = leaf blade color, LUP = leaf upper surface, LS = ligule shape.

CONCLUSION

Rejuvenation of 15 Yogyakarta local rice has regenerated new seeds consisting 13 cultivars that could produce >100 g of seeds per cultivar. The qualitative and quantitative characters can be used as a reference in plant breeding selection of new rice varieties. Visual color clustergram analysis showed that quantitative character could be grouped into three groups with days of maturity, plant height, number of grains per panicle, and grain weight as determining characters, while qualitative character can be grouped into two groups with grain color and flag-leaf attitude which can be used as reference character in selection.

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