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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Taufiq Hidayat R. S., Marjani, Nurindah, et al.

Secondary Characters Based Selection of Indonesian Kenaf (*Hibiscus cannabinus* L.) Germplasm for Developing Superior Varieties

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Abstract. Indonesia has released 13 varieties of kenaf derived from the refining of several introduced lines and hybridization. Lines selection was carried out to obtain lines that have certain superior characters. This study aimed to determine the agronomic characters that can be used as secondary characters for selection to support the developing process of new superior varieties. A total of 70 kenaf accessions from Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI) germplasm collection, was mainly introduced accessions from International Jute Organization (IJO). Primary data of the 16 quantitative morphological characters were observed based on the total accessions to be subjected for analysis using correlation, path analysis, and scatter plot. The characters of the first flower phase, 50% flower blooming, fiber harvest phase, plant height, diameter, and dry core weight had a significant and positive correlation coefficient with the dry weight of kenaf fiber. The dry weight of the core showed the best direct effect and total effect value, i.e. 0.88 and 0.84, respectively. Based on dry core and fiber weight characters, three distribution clusters of 70 kenaf accessions were obtained. In quadrant III, two accessions potentially produce the best dry fiber and they can be considered a source of parent in developing a new superior variety of kenaf.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is one of the natural fiber-producing plants with higher cellulose content. This plant can be used as raw material for various purposes, i.e. textile product, pulp and paper, feed raw materials, bioplastic and biocomposite industries [1], food raw material [2], bioenergy raw materials [3], and traditional medicine [4]. The kenaf development provides economic opportunities for fiber production. However, the fiber productivity has decreased. Based on world kenaf production data, it shows that kenaf production has reduced up to 19% from 269.2 thousand tons in 2012/2013 to 218.0 thousand tons in 2018/2019. Meanwhile, kenaf production in Indonesia in 2018/2019 only reached 3.1 thousand tons [5].

In an effort to develop kenaf, the problem encountered is the lack of superior varieties with high fiber production. Indonesia has released 13 varieties that were derived from the refining of several introduced lines and hybridization. In developing varieties through hybridization, individual and lines selections were carried out to obtain lines that have certain superiority characters. The development of superior varieties that are able to produce high fiber and can adapt to biotic and abiotic environmental conditions is obtained through the selection stage on germplasm or by selecting segregated populations. Kenaf genotype selection can be made directly by observing the yield obtained. However, the selection effectivity will be low because the environmental factors will give more remarkable effects than genetic factors [6]. Therefore, it is necessary to include the secondary characters

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observation, namely agronomic characters which related to high production. The genetic relationship between the yield and other characters can be estimated by calculating the value of the correlation coefficient.

Correlation is a method of determining the closeness of one character to another [7, 8]. Correlation analysis is mostly used to determine linear relationships between characters. In addition, an important and effective tool for the selection for the selection response, it depends upon many factors affecting the mutual relations between the various characters [9]. Although, correlation can help reveal the degree of linearity of a pair characters, the degree of correlation is unable to determine the relationship between direct and indirect effects to the outcome. This weakness can be overcome with path analysis.

Path analysis provides more information between variables such as sorting out the correlation coefficient into direct and indirect effects [10]. Generally, this approach is used in plant breeding to describe the reciprocal relationship between characters and yield components or the result of interactions between genotypes and the environment or cultivation system management [11]. Path analysis is able to explain the complex relationships between observed characters [12]. The results of path analysis help to formulate the basis for direct and indirect selection criteria. For example, the selection aimed at increasing certain desired characters but difficult to measure (such as production) with low heritability values, selection can be done indirectly on other characters. The effect of vegetative and generative characters on the yields is analyzed by path analysis, therefore it will lose information about the relationships between the vegetative characters and yield if the analysis results are only generative characters that have a significant effect [13].

Apart from the need for character selection to produce secondary characters, it is also necessary to select accessions as parents of hybridization in plant breeding. Selection of parents requires a grouping stage to obtain parents who have a distant kinship. A clustering can be performed based on two variables using a scatter plot that would provide an information of identified accessions. The scatter plot serves to test close relationships and determine the type of relationships between two variables. The first variable is obtained from the character of the yield production, such as dry fiber weight. The second variable comes from the result of secondary character selection using path analysis. The scatter plot shows herbal medicine characteristics of clusterization according to the sample type. After extraction, reducing the concentration in the residual powder shows a slight shift to the right, and a strong shift in the opposite direction for extracts. The shift intensity correspondence was predicted to be stronger for samples with a higher content of flowers [14].

The corn cobs weight can be used as a selection character because it produces high and positive direct and indirect effects on seed production and other characters [15]. The character of tuber weight could be used as a selection criterion for sweet potato clones with high beta-carotene levels based on heritability, genetic variability, correlation, and path analysis [16]. The characters of corn plant height, cobs length, and kernel row in cobs can be used as selection characters because they have a real and positive direct effect on the character of the yield components [17]. The plant height, base diameter, green weight, and stick weight exhibited highly a significant positive correlation with dry fiber yield that could be used as the selection criteria to enhance the yield of kenaf [18]. This study aimed to determine the characters in the generative growth phase that can be used as a secondary selection characters for the efficient developing process of new superior varieties.

MATERIALS AND METHODS

This study used 70 kenaf accessions as genetic resources from Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI) germplasm collection. Most of them were introduced accessions from International Jute Organization (IJO). Kenaf germplasm accessions were planted in the Sumberejo Experimental Station, ISCFRI in 2015. Each accession was planted in an experimental plot size (5×1) m^2 with 30 cm × 20 cm spacing. The agronomical traits were observed on 10 plant individuals randomly at the vegetative and generative phases. The primary data collection to determine correlation values, path analysis, and scatter plot of agronomy characters of the total kenaf was carried out in August 2020 until February 2021.

The variables observed were X_1 = leaf length, X_2 = leaf width, X_3 = petiole length, X_4 = first flower, X_5 = 50% flower blooming, X_6 = fiber harvest, X_7 = seed harvest, X_8 = plant height, X_9 = rootstock stem diameter, X_{10} = medium stem diameter, X_{11} = top stem diameter, X_{12} = core diameter, X_{13} = number of internodes, X_{14} = 1,000 seeds weight, X_{15} = dry core weight, and Y = dry fiber weight. The data from the observations were analyzed for correlation, path analysis and scatter plot (70 kenaf accessions). Correlation analysis between variables is carried out by calculating the value of a simple linear correlation coefficient based on the formula [19]:

$$r = \frac{\sum X_1 X_2}{\sqrt{(\sum X_1^2)(\sum X_2^2)}} \quad (1)$$

where r = correlation coefficient, X_1 and X_2 is the mean of the observed variables. Path analysis is carried out based on the following simultaneous equation [20]:

$$\begin{bmatrix} r_{11} & r_{12} & \ldots\ldots & r_{1p} \\ r_{21} & r_{22} & \ldots\ldots & r_{2p} \\ . & . & \ldots\ldots & . \\ . & . & \ldots\ldots & . \\ . & . & \ldots\ldots & . \\ r_{p1} & r_{p2} & \ldots\ldots & r_{pp} \end{bmatrix} \quad \begin{bmatrix} C_1 \\ C_2 \\ . \\ . \\ . \\ C_p \end{bmatrix} \begin{bmatrix} r_{1y} \\ r_{2y} \\ . \\ . \\ . \\ r_{py} \end{bmatrix} \quad (2)$$

R_x $\quad$ $\underline{C}$ $\quad$ R_y

R_x is the inter-independent variable correlation matrix in the multiple regression model with p independent variables which is a matrix with elements r_{xixj} (i,j = 1, 2,…, p). C is the path coefficient vector that shows the direct effect of each standardized independent variable, Z_i, on the dependent variable (standard regression coefficient value). R_y is the correlation coefficient vector between the independent variables X_i. i = 1, 2,…, p; the independent variable y. From the matrix equation, can be determined the path coefficient vector C, as follows [19]:

$$C = r_x^{-1} r_y$$

$$\begin{bmatrix} r_{11} & r_{12} & \ldots\ldots & r_{1p} \\ r_{21} & r_{22} & \ldots\ldots & r_{2p} \\ . & . & \ldots\ldots & . \\ . & . & \ldots\ldots & . \\ . & . & \ldots\ldots & . \\ r_{p1} & r_{p2} & \ldots\ldots & r_{pp} \end{bmatrix} \quad \begin{bmatrix} C_1 \\ C_2 \\ . \\ . \\ . \\ C_p \end{bmatrix} \begin{bmatrix} r_{1y} \\ r_{2y} \\ . \\ . \\ . \\ r_{py} \end{bmatrix} \quad (3)$$

R_x $\quad$ $\underline{C}$ $\quad$ R_y

r_x^{-1} is the inverse matrix of R_x and R_y is a vector correlation coefficient between independent variables X with the dependent variable Y. The direct effect of standardized independent variables on the dependent variable Y is measured by the line coefficient C_i. The indirect effect of the independent variable Z_i on the dependent variable Y through the independent variable Z_j (in the model) is measured by C_j. r_{ij}.

The path analysis model explains the error effect. The effect that cannot be explained by a model is entered as the effect of the error or residual which is measured by the formula [19]:

$$h\ (error) = \sqrt{1 - C^1 A} \quad (4)$$

Data analysis was conducted using Minitab Statistical Software version 15 for correlation analysis, path analysis, and scatter plot.

RESULTS AND DISCUSSIONS

Correlation of Agronomic Character to Dry Fiber Weight

Estimation of correlation analysis is intended to study the relationship pattern between agronomic characters and yields. In plant breeding, agronomic characters are more influenced by their environmental factors. The selection will be more effective if also done indirectly through a character that is positively correlated to the yield component.

TABLE 1. Correlation between several variables (X_i) and yield (Y).

	Variable (X_i)	Correlation coefficient value on yield (Y)
X_1	Leaf length	-0.182
X_2	Leaf width	-0.054
X_3	Petiole length	0.188
X_4	First flowering age	0.467
X_5	50% flowering age	0.474
X_6	Harvest of fiber	0.581
X_7	Harvest of seeds	-0.107
X_8	Plant height	0.650
X_9	Rootstock stem diameter	0.150
X_{10}	Medium stem diameter	0.171
X_{11}	Top stem diameter	-0.004
X_{12}	Core diameter	0.404
X_{13}	Number of internodes	0.280
X_{14}	1,000 seeds weight	0.082
X_{15}	Dry core weight	0.916

The correlation coefficient between agronomic characters and yield are presented in Table 1. The correlation analysis between several variables (variable, X_i) and yield (Y) on 70 kenaf accessions showed that there were six variables that were positively correlated with the yield, namely the first flowering age (r = 0.467), 50% flowering age (r = 0.474), fiber harvest (r = 0.581), plant height (r=0.650), core diameter (r = 0.404), and dry core weight (r = 0.916). This indicated that the six characters have a close relationship and were able to increase the value of the outcome variable. Thus, the six characters can be used as a selection character for 70 kenaf accessions in developing new superior varieties.

The significant and positive correlation coefficient value showed that the greater the variable value, the greater the yield [19]. Conversely, the smaller the variable value the lower the yield. The significant and negative correlation coefficient values indicated the greater the variable value the lower the yield obtained. Conversely, the smaller the variable value, the greater the yield. The significant positive correlations characters indicate that these characters are very important and can be considered as indirect selection characters in increasing yield components [21]. The similar results were also obtained on green biomass weight, plant height, basal diameter, and days to 50% flowering as the selection parameters to produce jute varieties with acceptable yield [22].

Path analysis to Obtain Variables Affecting to Yield Production

The fiber dry weight character illustrates the yield potential of kenaf. The yield production characters can be in the form of biological or agronomic yields. Several variables that are positively correlated with the yield (Table 1) could be used as the character for the selection of kenaf accessions. Genetically, the correlation between agronomic characters is also caused by pleiotropy and linkage phenomena. Thus, path analysis is needed to obtain more detailed information regarding the influence of agricultural characters on the main character. In addition, through path analysis, it can also be determined which characters directly or indirectly effect on the yield components of kenaf.

The path analysis results (Table 2) showed that the 50% flowering age (P_{X5} = 0.79), plant height (P_{X8} = 0.13), core diameter (P_{X12} = 0.10), and dry core weight (P_{X15} = 0.88) possessed a direct positive effect to the yield. However, the dry core weight has the highest direct and total effect, namely 0.88 and 0.84, compared to other agronomic characters. These results suggest that the dry core weight character is very influential on the yield of kenaf and can be used as a more effective direct selection character. Grain weight of main panicle also greatly affected the yield component of wheat, allowing its potential to be used to select directly genotypes of wheat since it

produced a highly value of direct and total effect, i.e. amounted to 0.837 and 0.994, respectively [6]. The difference in direct genetic and phenotypic effects on seed yield is due to environmental effect on these characters [23].

TABLE 2. The direct, indirect, and total effect of variables on the yield.

Agronomic characters	Direct effect	The coefficient value of the indirect effect of the variables						Total effect
		X_4	X_5	X_6	X_8	X_{12}	X_{15}	
X_4	-0.82460		0.78108	-0.10281	0.08906	0.06882	0.43089	0.44242
X_5	**0.78500**	-0.82048		-0.10329	0.08733	0.06842	0.38447	0.40145
X_6	-0.15866	-0.53434	0.51104		0.08096	0.03995	0.53511	0.47405
X_8	**0.13272**	-0.55331	0.51653	-0.09678		0.05583	0.57890	0.63389
X_{12}	**0.09988**	-0.56815	0.53773	-0.06346	0.07419		0.33630	0.41649
X_{15}	**0.87579**	-0.40570	0.34462	-0.09694	0.08773	0.03835		**0.84384**

X_4 = the first flowering, X_5 = 50% flower blooming, X_6 = fiber harvest, X_8 = plant height, X_{12} = core diameter, X_{15} = dry core weight.

The character of the first flowering age has a negative direct effect value and great characters, indicating its contribution as a negative dominant effect to the character of dry fiber weight. Thus, the character of the age of the first flower can be used as an indicator of indirect selection of fiber dry weight. The angular character of the stem leaves gave a negative direct effect value (-1.725), indicating that this character can be used as a character for indirect selection of catechin content on tea without considering the existence of an indirect effect [24]. The results of path analysis can be made a path diagram as a unidirectional causal relationships model. Based on the path analysis in Table 2, some variables affect the yield production kenaf fiber (Fig. 1).

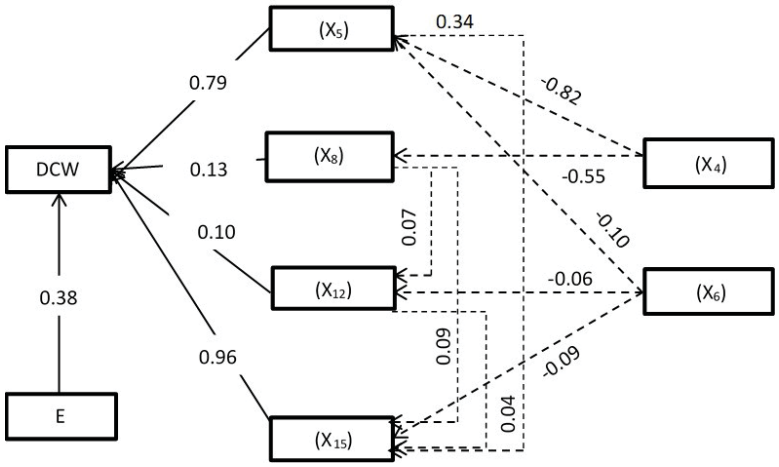

FIGURE 1. Path diagram of variables that affect the yield production of kenaf. X_4 = the first flowering, X_5 = 50% flower blooming, X_6 = fiber harvest, X_8 = plant height, X_{12} = core diameter, X_{15} = dry core weight, DCW = dry core weight, E = error.

The variables that have a low coefficient of a direct effect on the yield production is necessary to be given attention to the value of the indirect effect on the character of the yield to other characters. The variables that do not affect the yield to other variables can be selected which have an indirect effect coefficient value >0.09 [19]. However, based on Fig. 1, the first flower and fiber harvest had low indirect effects for all agronomic characters used. Meanwhile, 50% flowering age had a large indirect effect on the dry core weight, which was 0.34. It indicates that this character can be used as an indirect selection parameter at 70 accessions of kenaf.

The error effect in the analysis of variance determines independent variables can explain the dependent variable. In Fig. 1, an error effect of 0.38 (38%) indicates that 62% of the variability in the yield components can be explained by the component factors. In rice grain study, high error effect coefficient was 0.64 and there were other variables which determined yield and were not yet explained in that study [25]. The direct effects of the variables analyzed

were inferior to the error variable effect (0.57), indicating that the variables analyzed in this study are not the main determinants of grain yield, although they have influence on this trait [26].

Distribution of 70 Kenaf Accessions Based on Dry Core and Fiber Weight Characters

The path analysis in Fig. 1 showed that dry core weight variable could be used as a secondary selection character. Furthermore, a selection was carried out using a scatter plot on 70 kenaf accessions with variable dry core weight (X) and dry fiber weight (Y) (Fig. 2).

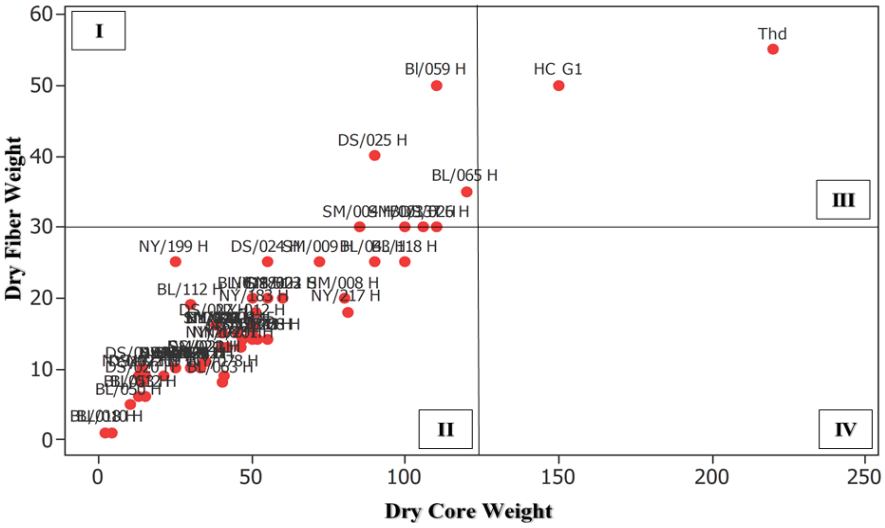

FIGURE 2. Distribution of 70 kenaf accessions based on dry core weight and dry fiber weight characters.

The scatter plot shows the formation of four quadrants which have different distribution of kenaf accessions. The quadrant I preferred cluster that has a high dry fiber weight and a low dry core weight. The quadrant II showed the clustered accessions that had a low dry core weight and dry fiber weight. The quadrant III was accessions with high dry core weight and dry fiber weight. Meanwhile, quadrant IV was clustered accessions with high dry core weight but a low dry fiber weight. Based on these two characters, three distribution clusters of 70 kenaf accessions were obtained. The quadrant I consisted of 7 kenaf accessions, quadrant II clustered 61 accessions, and quadrant III comprised 2 accessions. Based on the scatter plot of morphological characters, three clusters were generated. Of the total 32 kenaf genotype, cluster 1, 2, and 3 contained 11, 16, and 5 genotypes, respectively [27]. Distributed accessions in quadrant III were those that have the potential to produce higher fiber. Accessions HC G1 and Thd were capable of producing the best dry fiber and potential to be used as material for developing new superior variety of kenaf. According to the analysis between seed production and yield components of kenaf, two genotypes with high seed production and fiber yield potential were identified for their promising performance. AU-75 and Ifaken 100 were recorded as the genotypes with higher yield of 1,454 and 1,419 kg/ha, respectively [28]. Overall. based on the criteria for resistance to Fusarium wilt, one accession (FJ/017) was found a highly resistant, while the remaining 14 accessions were categorized as resistant that could be used further as resistant genetic sources in developing new varieties [29].

CONCLUSION

Agronomic characters of kenaf have a positive correlation coefficient with the dry weight characters, i.e. the first flower phase, 50% flower blooming, fiber harvest phase, plant height, core diameter, and dry core weight. The 50% flowering age, plant height, core diameter, and dry core weight have a positive direct effect value. To develop superior kenaf varieties, dry core weight is the best character to be used as a direct selection parameter because it has the best direct and total effect value, i.e. 0.88 and 0.84, respectively. The 50% flowering age can be used as an indirect selection character because it has the best indirect effect value on dry core weight. Three distribution

clusters of 70 kenaf accessions were successfully generated based on dry core and fiber weight characters. Two accessions showed their potential to produce dry fiber and can be considered as a source of parent in developing new superior variety of kenaf.

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