Morphological characteristics, chemical and genetic diversity of kenaf (*Hibiscus cannabinus* L.) genotypes

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Abstract The kenaf plant is used widely as food and in traditional folk medicine. This study evaluated the morphological characteristics, functional compounds, and genetic diversity of 32 kenaf cultivars from a worldwide collection. We found significant differences in the functional compounds of leaves from all cultivars, including differences in levels of chlorogenic acid isomer (CAI), chlorogenic acid (CA), kaempferol glucosyl rhamnoside isomer (KGRI), kaempferol rhamnosyl xyloside (KRX), kaempferitrin (KAPT) and total phenols (TPC). The highest TPC, KAPT, CA, and KRX contents were observed in the C22 cultivars. A significant correlation was observed between flowering time and DM yield, seed yield, and four phenolic compounds (KGRI, KRX, CAI, and TPC) ($P < 0.01$). To assess genetic diversity, we used 80 simple sequence repeats (SSR) primer sets and identified 225 polymorphic loci in the kenaf cultivars. The polymorphism information content and genetic diversity values ranged from 0.11 to 0.79 and 12 to 0.83, with average values of 0.39 and 0.43, respectively. The cluster analysis of the SSR markers showed that the kenaf genotypes could be clearly divided into three clusters based on flowering time. Correlations analysis was conducted for the 80 SSR markers; morphological, chemical and growth traits were found for 15 marker traits (corolla, vein, petal, leaf, stem color, leaf shape, and KGRI content) with significant marker-trait correl-

ations. These results could be used for the selection of kenaf cultivars with improved yield and functional compounds.

Keywords Kenaf, Morphological characteristics, Phenolic compounds, SSR markers, Correlation analysis

Abbreviations

SSR	Simple sequence repeats
UPLC	Ultra-high performance liquid chromatography
CAI	Chlorogenic acid isomer
CA	Chlorogenic acid
KGRI	Kaempferol glucosyl rhamnoside isomer
KRX	Kaempferol rhamnosyl xyloside
KAPT	Kaempferitrin
TPC	Total phenols

Introduction

Kenaf (*Hibiscus cannabinus* L.), an annual herbaceous crop of the family Malvaceae ($2n = 36$), is a short-day annual herbaceous plant that tolerates a broad range of soil types and climates (Dempsey 1975). Kenaf has traditionally been used to make rope but the plant is used in many different ways, including as pulp, potting media, a bioplastic, and cellulosic biofuel (Alexopoulou et al. 2013). Recently, kenaf being used valuable dual-purpose crop for fiber and medicinal. Its leaves contain large amounts of a variety of compounds including polyphenols (Jin et al. 2013; Ryu et al. 2017a). The most common phenolic compounds in kenaf are the kaempferol glycoside, caffeic acid, myricetin glycoside, and *p*-hydroxybenzoic acid (Ryu et al. 2017a). In ayurvedic medicine, the kenaf are used for bilious, blood, diabetes, biliousness, coughs and throat disorders (Alexopoulou et al. 2013; Ryu et al. 2017a).

Kenaf has erect, tall plants with few or no branches that

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may reach 3 ~ 5 m in height depending on climatic conditions (Alexopoulou et al. 2013; Ryu et al. 2013). Its leaves may be non-lobed (entire), shallow-lobed, or deeply lobed (palmate). Kenaf flowers, are bell-shaped, open with five petals, and have colors ranging from light cream to dark purple (Alexopoulou et al. 2013). Korean Kenaf cultivars are divided into three maturation groups depending on flowering date; early-maturing, mid-late maturing, and late maturing. Early-maturing groups mature in 70 ~ 80 days after seeding and it allow seed harvests, but the lower biomass. Late-maturing groups grow vegetatively for 130 ~ 140 days and yield significantly higher biomass. However, late maturation reduces seed quality (Ryu et al. 2016a; Jeong et al. 2017). Breeding selection of new kenaf cultivars is the most effective measure to increase biomass and seed yields per unit area (Alexopoulou et al. 2013). Mutation breeding has the merits of creating new mutant characteristics and adding only very few traits without disturbing the other characteristics of a cultivar (Kang et al. 2016; Ryu et al., 2017b). Mutagenic agents, such as radiation, can be used to induce mutations and generate genetic variants from which desired mutants may be selected (Visser et al. 1971). This process offers the possibility of inducing desirable attributes that either have not developed in nature or have been lost during evolution. In crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time or color, and resistance to pathogens (IAEA 2016; Kang et al. 2016; Ryu et al. 2016b; Ryu et al. 2017b).

To cultivate kenaf in a tropical environment, it is necessary to evaluate the available genotypes in terms of their morphological characters (Faruq et al. 2013). Moreover, the identification of genetic relationships and diversity is one of the most important factors in the selection of cultivars for a breeding program (Jeong et al. 2017; Thakur et al. 2017). Simple sequence repeats (SSRs) are one of the most commonly used types of DNA molecular markers for revealing genetic diversity among genotypes (Varshney et al. 2005). Knowledge of level of genetic diversity, morphological and chemical relationships among genotypes essential for establishing, managing, and ensuring the long-term success of crop-improvement programs (Varshney et al. 2005; Jeong et al. 2017; Thakur et al. 2017). In this study, we compared morphological, phytochemical, and genetic characteristics among kenaf cultivars from eight countries (Bangladesh, China, India, Iran, Italy, Korea, USA, and Russia). We examined morphological and chemical characteristics to establish relationships, and compared these relationships to genetic relationships. In addition, we used SSR markers to evaluate genetic diversity, and demonstrated significant associations

between the SSR markers and morphological and phytochemical characteristics.

Materials and Methods

Plant materials

Thirty-two cultivars were studied. These included six mutant genotypes (Jangdae, Jeokbong, RS1, RS2, WFM1-2 and Baekma), two original cultivars (Jinju and C14), and 24 accessions collected worldwide from the Genebank of Rural Development Administration (RDA) in Korea and the Bangladesh Jute Mills Corporation (BJC). The Jangdae cultivar combines high biomass and seed yield and has been tested by the Korea Seed and Verity Service (KSVS). Five of the morphological mutants exhibiting changes in flower color (Baekma, WFM1-2) and stem color (Jeokbong, RS1, RS2) were derived from the same kenaf cultivar (C14) introduced from Italy. The whole plant and leaves of the genotypes were harvested at the flowering time of each genotype for dry matter (DM) yield and phenolic compounds analysis. Seeds were planted in plots (3 × 4.2 m) with row spacings of 20 and 60 cm, respectively. Fertilizer (N:P:K 4:2:2 w/w/w) was applied at 550 kg/ha shortly after seeding. Manure was spread before planting, but the plants were not fertilized after planting. The experiment was conducted at the Korea Atomic Energy Research Institute.

Morphological and agronomic characteristics

All genotypes were planted on mid-May and measured for morphological and agronomic characteristics during 2012 to 2015. The cultivars were divided into three different flowering groups, early flowering (70 ~ 84 day after seeding), mid-late flowering (101 ~ 134 day after seeding), and non-flowering. We measured the morphological traits of each sampled individual: leaf shape (palmate 1, entire 2), leaf color (green 1, purple 2), branch color (green 1, brown 2, purple 3), vein color (green 1, purple 2), hypocotyl colors (green 1, purple 2), stem colors (brown 1, green 2, purple 3) corolla color (green 1, purple 2, non 3), and petal colors (Ivory 1, white 2, non 3).

UPLC analysis

Phenolic compounds were analyzed using a *ultra-high performance liquid chromatography* (UPLC) system (CBM-20A, Shimadzu Co., Kyoto, Japan) with two gradient pump systems

(LC-30AD, Shimadzu), a UV-detector (SPD-MB0A, Shimadzu), an auto sample injector (SIL-30AC, Shimadzu), and a column oven (CTO-30A, Shimadzu). Separation was achieved on an XR-ODS column (3.0 × 100 mm, 1.8 µm, Shimadzu, Japan) using a linear gradient elution program with a mobile phase containing solvent A (0.1%, v/v, trifluoroacetic acid in distilled deionized water) and solvent B (0.1%, v/v, trifluoroacetic acid in acetonitrile). Samples for UPLC analysis of phenolic compound contents were ground using a grinder immediately prior to analysis. All samples were ground to achieve a particle size that would pass through a 500 µm sieve. For UPLC analysis, ground samples (1 g) were extracted in 5 mL water for 16 h and filtrated through a 0.45 µm membrane filter. The phenolic compounds were separated using the following gradient: 0 ~ 5 min, 10 ~ 15% B; 5 ~ 10 min, 15 ~ 20% B; 10 ~ 15 min, 20 ~ 30% B; 15 ~ 20 min, 30 ~ 50% B; 20 ~ 25 min, 50 ~ 75% B; 25 ~ 30 min, 75 ~ 100% B; 30 ~ 32 min, 100 ~ 5% B; 32 ~ 35 min, 5 ~ 0% B. The phenolic compounds were detected at 280 nm. Identification of kaempferitrin (KAPT) was determined based on the retention times of commercial standards (UV spectrum) and the Chlorogenic acid isomer (CAI), Chlorogenic acid (CA), Kaempferol glucosyl rhamnoside isomer (KGRI), Kaempferol rhamnosyl xyloside (KRX) were identified using retention time, UV-visible spectral characteristics.

DNA extraction and simple sequence repeat analysis

DNA was extracted from young leaves of each cultivar using the cetyltrimethylammonium bromide (CTAB) method. A total of 70 EST-SSR primers set were synthesized based on published sequence information (Jeong et al. 2017) and 10 genomic SSR primer sets were obtained from the US National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) *Hibiscus cannabinus* database. SSR sequence PCR reactions were carried out in 25 µL of a mixture containing 20 ng genomic DNA, 10 pmol primer, 1 unit *Taq* polymerase, 10× PCR reaction buffer, and 0.2 mM dNTP. The PCR cycling conditions were as follows: 94°C for 5 min (initial denaturation), followed by 40 cycles at 94°C for 45 s (predenaturation), 55°C for 45 s (annealing), 72°C for 90 s (extension), with a final 7 min extension at 72°C and cooling to 4°C. The samples were fractionated using a LabChip GX electrophoresis system (Caliper Life Science, USA).

Statistical analysis

The chemical analysis data were subjected to analysis of

variance using a multiple comparisons method with the statistical software package SPSS version 12 (SPSS Institute, USA). Differences were determined to be significant at $p < 0.05$. When the treatment effect was significant, means were separated using Duncan's multiple range tests. The correlation coefficients between the morphological and agronomics characteristics and the phenolic compound contents were obtained using Pearson's correlation coefficients ($P < 0.01$) to examine the degree among kenaf genotypes.

All of the SSR bands were scored as 0 or 1 for the absence or presence of the band, respectively. The polymorphism information content (PIC), mean gene diversity (GD), and Shannon's information index (SI) for each SSR marker were calculated using Power Marker Ver. 3.25.

The morphological, chemical and genetic dendrogram were constructed using the phylogenetic tree using the Neighbor Joining method (Saitou and Nei 1987) with the statistical software package Power Marker Ver. 3.25.

We determined correlation between morphological characteristics, phenolic compound contents and SSR markers using TASSEL 3.0.1 software (Bradbury et al. 2007). We performed three tests for significance. First, the Q general linear model (GLM) was used on the chosen Q-matrix derived from STRUCTURE with 10,000 permutations to test marker significance and determine the experiment-wise P value for each marker's significance. Second, the Q-mixed linear model (Q-MLM) method was used to determine a kinship matrix. SPAGeDi was used to calculate kinship (K) coefficients.

Results

Morphological and agronomic characteristics

Selected results of the evaluation of morphological characteristics are presented in Table 1 and Figure 1. The leaf shapes of the kenaf cultivars were divided into two types: 11 cultivars with entire and 21 cultivars with palmate leaves. All kenaf cultivars had green leaves, except for the Jeokbong cultivar, which had greenish purple leaves. The colors of branches and stems were green, brown, or purple. Veins, hypocotyls, and corollas were green or purple. Finally, flower petals were ivory (18 genotypes) or white (2 genotypes), while 12 genotypes were non-flowering.

The growth characteristics of the kenaf genotypes are shown in Table 2. We observed significant differences for most of the growth and yield characteristics including plant height, fresh mater yield (FM) and seed yield. The plant

Table 1 The origin and morphological characteristics of kenaf genotypes used in this study

No.	Name	Origin	Source (cultivar or accession number)	Flowering date	Leaf shape	Leaf color	Branch color	Vein color	Hypocotyl color	Stem color	Corolla color	Petal color
1	C9	Russia	IT202789 ^z	Early	Palmate	Green	Green	Green	Purple	Brown	Green	Ivory
2	C10	India	IT202790	Early	Palmate	Green	Green	Green	Green	Brown	Green	Ivory
3	C11	Iran	IT202791	Early	Entire	Green	Brown	Green	Purple	Brown	Green	Ivory
4	C12	Italy	IT202792	Early	Palmate	Green	Brown	Green	Purple	Brown	Green	Ivory
5	C13	Russia	IT202793	Early	Palmate	Green	Brown	Green	Purple	Brown	Green	Ivory
6	C14	Italy	IT202794	Early	Palmate	Green	Brown	Green	Purple	Brown	Green	Ivory
7	C15	Africa	IT202795	Early	Palmate	Green	Green	Green	Purple	Brown	Green	Ivory
8	C16	China	IT202796	Early	Palmate	Green	Green	Green	Green	Brown	Green	Ivory
9	C17	China	IT202797	Early	Palmate	Green	Green	Green	Purple	Brown	Green	Ivory
10	C18	China	IT202798	Early	Palmate	Green	Brown	Green	Green	Brown	Green	Ivory
11	C19	India	IT202799	Early	Palmate	Green	Brown	Green	Purple	Brown	Green	Ivory
12	C20	India	IT202800	Early	Palmate	Green	Brown	Green	Green	Brown	Green	Ivory
13	C22	Russia	IT202802	Early	Palmate	Green	Brown	Green	Purple	Brown	Green	Ivory
14	RS1	C14	Mutant line	Early	Entire	Green	Purple	Purple	Purple	Purple	Purple	Ivory
15	RS2	C14	Mutant line	Early	Palmate	Green	Purple	Purple	Purple	Purple	Purple	Ivory
16	Jeokbong	C14	Cultivar	Early	Entire	Purple	Purple	Purple	Purple	Purple	Purple	Ivory
17	Baekma	C14	Cultivar	Early	Entire	Green	Green	Green	Green	Green	Green	White
18	WFM1-2	C14	Mutant line	Early	Entire	Green	Brown	Green	Purple	Brown	Green	White
19	Jangdae	Jinju	Cultivar	Mid-late	Palmate	Green	Green	Green	Green	Green	Green	Ivory
20	Jinju	Unknown	Accession	Mid-late	Entire	Green	Green	Green	Green	Green	Green	Ivory
21	Hongma300	China	Cultivar	Late	Palmate	Green	Brown	Green	Green	Brown	- ^x	Ivory
22	Hongma74-3	China	Cultivar	Late	Palmate	Green	Green	Green	Purple	Green	-	Ivory
23	Everglades41	USA	Cultivar	Late	Palmate	Green	Brown	Green	Purple	Brown	-	Ivory
24	ACC3748	Unknown	BJC ^y	Late	Entire	Green	Brown	Green	Green	Brown	-	Ivory
25	ACC4111	Unknown	BJC	Late	Entire	Green	Brown	Green	Green	Brown	-	Ivory
26	ACC4153	Unknown	BJC	Late	Entire	Green	Brown	Green	Green	Brown	-	Ivory
27	ACC4139	Unknown	BJC	Late	Palmate	Green	Brown	Green	Green	Brown	-	Ivory
28	ACC4443	Unknown	BJC	Late	Palmate	Green	Purple	Purple	Purple	Purple	-	Ivory
29	ACC4751	Unknown	BJC	Late	Entire	Green	Brown	Green	Green	Brown	-	Ivory
30	ACC4985	Unknown	BJC	Late	Palmate	Green	Green	Green	Green	Green	-	Ivory
31	ACC5014	Unknown	BJC	Late	Palmate	Green	Green	Green	Purple	Green	-	Ivory
32	ACC5072	Unknown	BJC	Late	Entire	Green	Green	Green	Green	Green	-	Ivory

^zIT No. : Genebank, National Agro Biodiversity Center, Rural Development Administration, ^yBJC: Bangladesh Jute Mills Corporation

^xnon-flowering

height of all the genotypes ranged from 234.4 to 415.9 cm. The Hongma300 had the highest FM yield and lowest yield was found in C20 genotype. The highest seed yield was recorded for the Jangdae cultivar and twelve late flowering genotypes were impossible seed harvest.

We generated a phylogenetic tree using the Neighbor Joining method (Saitou and Nei 1987) for morphological

and agronomic characteristics relationships (Fig. 2A). The kenaf genotypes were divided into six groups based on phylogenetic tree the morphological and agronomic characteristics. Group 1 consisted of two genotypes (C10 and C16) with concurrent flowering date and morphological phenotype (palmate and green leaf, green vein, corolla and hypocotyl color, brown stem, ivory flower). Group 2 contained three

Fig. 1 Comparison of morphological characteristics of kenaf genotypes. A: Entire leaf, B: palmate leaf, C: leaf color (purple), D: Ivory petal, E: white petal, F: branch color (purple), G: branch color (brown), H: branch color (green), I: Hypocotyl color (green), J: Hypocotyl Color (purple), K: stem color (green), L: stem color (brown), M: stem color (purple)

Table 2 Growth characteristics of kenaf genotypes

Genotype	Plant height (cm)	Dry matter yield (ton/ha)	Seed yield (kg/ha)
C9	247.5±05.2 ^k	74.8±1.6 ^h	588.7±06.0 ^f
C10	238.8±08.7 ^l	75.3±2.7 ^h	514.2±09.2 ^j
C11	274.5±03.6 ^{gh}	77.7±1.1 ^{gh}	636.5±04.3 ^c
C12	272.5±01.6 ^b	76.0±0.5 ^{gh}	652.3±02.1 ^d
C13	252.7±01.7 ^k	74.0±0.5 ^h	538.5±01.7 ^{hi}
C14	268.1±02.9 ^h	76.0±0.8 ^{gh}	641.0±03.3 ^{de}
C15	276.9±02.9 ^{gh}	76.7±0.8 ^{gh}	669.1±03.3 ^c
C16	257.7±16.6 ^j	71.9±4.6 ^{hi}	545.1±17.9 ^h
C17	254.3±17.4 ^j	71.9±5.0 ^{hi}	566.2±20.3 ^g
C18	253.6±24.6 ^j	69.9±6.8 ^{hij}	457.1±23.4 ^k
C19	231.2±10.2 ^l	68.4±3.0 ^{hij}	426.9±09.4 ^l
C20	248.2±11.8 ^k	63.6±3.4 ^l	412.1±09.3 ^m
C22	234.4±05.6 ^l	65.9±1.5 ^j	325.1±03.7 ⁿ
RS1	261.6±00.4 ⁱ	70.2±0.0 ^{hi}	536.9±00.2 ^{hi}
RS2	255.8±03.8 ^j	69.4±1.0 ^{hij}	529.1±03.8 ⁱ
Jeokbong	268.9±09.3 ^h	73.1±2.5 ^h	579.5±09.8 ^f
Backma	288.4±01.1 ^{fg}	80.7±0.3 ^g	686.8±01.2 ^b
WFM1-2	293.3±02.0 ^f	80.7±0.6 ^g	687.0±02.4 ^b
Jangdae	345.8±11.8 ^{de}	117.0±4.0 ^f	847.5±14.2 ^a
Jinju	350.5±08.9 ^{de}	125.7±3.2 ^e	180.0±02.2 ^o
Hongma300	406.2±03.4 ^{ab}	144.1±1.2 ^{ab}	0.0 ^p
Hongma74-3	394.3±02.1 ^b	139.9±0.8 ^{bc}	0.0 ^p
Everglades41	415.9±03.7 ^a	148.1±1.3 ^a	0.0 ^p
ACC3748	369.5±02.8 ^c	133.6±1.0 ^d	0.0 ^p
ACC4111	352.4±08.9 ^d	139.9±3.5 ^{bc}	0.0 ^p
ACC4153	357.7±02.9 ^{cd}	138.6±3.3 ^c	0.0 ^p
ACC4139	385.4±03.6 ^b	134.6±1.2 ^{cd}	0.0 ^p
ACC4443	390.1±02.4 ^b	133.1±0.8 ^d	0.0 ^p
ACC4751	382.4±03.4 ^{bc}	136.0±1.2 ^c	0.0 ^p
ACC4985	338.2±05.9 ^e	135.9±2.3 ^c	0.0 ^p
ACC5014	365.6±08.6 ^{cd}	138.8±3.3 ^c	0.0 ^p
ACC5072	352.5±03.0 ^d	138.1±1.2 ^c	0.0 ^p

The letters above each point indicate a significant difference at the 5% level (Duncan's multiple range tests, n=3)

Fig. 2 Dendrogram showing the phenotypic relationship among the kenaf genotypes based on Pearson's correlation coefficients generated by morphological traits

genotypes (C18, C20 and C22) with same flowering date, leaf shape, leaf, branch, vein, stem, corolla and petal colors. Group 3 contained 9 early flowering genotypes (C9, C11, C12, C13, C14, C15, C17, C19 and WFM1-2). Group 5 included three purple stem mutant (Jeokbong, RS1 and RS2) and ACC4443 genotypes. Group 5 consists of 11 late flowering genotypes (Hongma300, Hongma74-3, Everglades41, ACC3748, ACC4111, ACC4153, ACC4139, ACC4751, ACC4985, ACC5014 and ACC5072). Group 6 included two mutant cultivars (Jangdae and Baekma) and Jinju with leaf, branch, vein, hypocotyl, stem and collroa were all green.

Phenolic compounds

The phenolic compound contents of the kenaf cultivars are shown in Table 3 and Figure 3. Five compounds, chlorogenic acid isomer (CAI), chlorogenic acid (CA), kaempferol glucosyl rhamnoside isomer (KGRI), kaempferol rhamnosyl xyloside (KRX), kaemperitrin (KAPT) were isolated. Significant differences in phenolic compound contents were observed among cultivars. The CAI content for all genotypes ranged from 18.0 to 64.0 mg/100 g. The ACC5072 genotypes had contained higher levels of CAI compounds than other genotypes. The highest CA content was observed in C22 (153.8 mg/100 g), and lowest levels were found in C20 (64.4 mg/100 g). The KGRI levels of all genotypes ranged from 29.8 mg/100 g for C9 to 108.6 mg/ 100 g for ACC4111. The KRX contents

ranged from 8.7 to C22 mg/100 g, with the highest amounts being in the C22. The KAPT content ranged from 97.8 to 306.1 mg/100 g. The highest KAPT content was observed in cultivar C22, and the lowest values were found in C9 (Fig. 3). The total polyphenol content (TPC) differed significantly among the cultivars, with the highest level found in C22 (766.8 mg/100 g⁻¹). The C9 cultivar had the lowest content (317.0 mg/100 g⁻¹).

Analysis of the phylogenetic tree showed that the 32 kenaf genotypes divided into three groups with the exception of the C13 genotypes, based on the six phenolic compounds (Fig. 2B). Group 1 included eleven early flowering genotypes (C9, C11, C15, C16, C17, C18, C20, Jeokbong, RS1, RS2 and Baekma). Group 2 included four early flowering genotypes (C10, C12, C22 and WFM1-2), two mid-late flowering genotypes (Jangdae and Jinju) and eleven late flowering genotypes (Hongma300, Hongma74-3, Everglades41, ACC3748, ACC4111, ACC4153, ACC4139, ACC4751, ACC4985, ACC5014 and ACC5072). Group 3 contained two genotypes (C19 and C16) with same morphological phenotype. C13 genotype was not belonging to any groups.

Pearson's correlation coefficients based on average quantified values for morphology and phenolic compound data are shown in Table 4. KGRI, KRX and TPC contents were correlated with flowering date, corolla color and petal color. CAI content was correlated with flowering date. Plant height, DM yield and seed yield were correlated with flowering

Table 3 Phenolic compound constituents in different kenaf genotypes

Genotypes	CAI ^z	CA ^y	KGRI ^x	KRX ^w	KAPT ^v	TPC ^u
C9	27.0±4.8 ^c	69.8±0.2 ^{ij}	29.8±4.2 ^h	11.1±2.2 ^{gh}	97.8±1.7 ^t	317.0±18.8 ^l
C10	27.7±5.0 ^c	90.8±6.9 ^{de}	45.1±5.6 ^g	15.8±1.7 ^{ef}	152.1±2.5 ^l	451.6±28.1 ^e
C11	26.7±4.1 ^c	77.6±3.2 ^{gh}	41.1±5.6 ^g	14.5±2.2 ^{fg}	136.4±2.0 ^p	387.9±20.5 ⁱ
C12	33.5±5.0 ^c	96.3±3.8 ^d	69.8±9.6 ^{cd}	21.5±3.1 ^c	180.9±0.9 ⁱ	531.9±22.2 ^d
C13	30.7±4.9 ^d	80.7±2.5 ^{fg}	48.9±6.7 ^f	16.5±2.5 ^e	140.6±1.8 ^o	411.2±19.3 ^h
C14	26.2±4.3 ^c	76.8±2.9 ^{hi}	45.2±5.3 ^g	16.5±2.0 ^c	146.0±2.5 ^m	409.4±21.2 ^h
C15	23.2±3.4 ^{fg}	80.2±3.6 ^{fg}	47.0±6.5 ^f	15.7±3.6 ^{ef}	127.3±0.9 ^q	387.7±13.5 ⁱ
C16	20.9±3.5 ^g	66.4±4.5 ^{lm}	33.7±4.5 ^g	10.6±0.5 ^{hi}	112.4±0.8 ^r	332.0±16.3 ^k
C17	25.5±3.8 ^c	69.0±2.7 ^{ij}	35.3±4.9 ^g	12.8±2.8 ^g	100.6±0.1 ^t	330.3±13.0 ^k
C18	27.4±4.3 ^c	76.7±0.1 ^{ih}	39.0±5.2 ^g	14.4±1.9 ^{fg}	123.8±1.3 ^q	376.1±18.3 ^j
C19	31.4±5.3 ^d	78.8±1.6 ^{fg}	43.5±5.3 ^g	16.9±2.8 ^{ef}	150.8±1.2 ^l	410.8±19.7 ^h
C20	26.9±4.1 ^c	60.4±2.0 ^m	32.7±4.4 ^g	11.9±2.1 ^{gh}	106.2±0.5 ^s	318.8±13.3 ^l
C22	18.0±1.8 ^g	153.8±10.7 ^a	98.9±14.6 ^{ab}	33.2±3.1 ^a	306.1±1.1 ^a	766.8±47.9 ^a
C14RS1	30.8±4.0 ^d	74.1±0.5 ⁱ	37.0±5.9 ^g	10.0±0.1 ^{hi}	98.7±3.0 ^t	364.8±16.8 ^j
C14RS2	31.5±4.9 ^d	73.4±1.3 ⁱ	36.9±5.4 ^g	13.1±2.4 ^{fg}	113.4±0.6 ^r	280.4±8.3 ^m
Jeokbong	7.4±0.3 ^h	19.0±0.8 ⁿ	43.3±5.7 ^g	10.9±2.5 ^{hi}	139.1±5.3 ^o	371.8±9.5 ^j
Baekma	24.0±1.6 ^{ef}	67.2±9.7 ^{kl}	33.4±5.8 ^g	9.1±0.0 ⁱ	146.2±2.9 ^m	364.2±1.0 ^j
WFM1-2	22.0±1.5 ^f	64.9±12.1 ^{lm}	32.3±6.9 ^g	8.7±0.3 ⁱ	194.6±2.6 ^h	441.3±4.8 ^f
Jangdae	26.6±3.7 ^c	88.3±8.8 ^{de}	75.7±9.6 ^c	16.3±2.3 ^{ef}	145.1±4.0 ^m	504.5±11.4 ^d
Jinju	32.8±4.5 ^c	91.5±1.1 ^{de}	86.3±10.3 ^b	21.5±4.5 ^c	191.0±2.8 ^h	546.5±11.1 ^d
Hongma300	26.4±3.5 ^c	88.8±7.9 ^d	75.9±9.2 ^c	16.3±2.2 ^{ef}	146.6±2.4 ^m	444.2±8.5 ^f
Hongma74-3	25.5±3.2 ^c	94.3±5.6 ^d	79.2±10.2 ^c	18.9±2.1 ^{de}	174.7±3.4 ^j	492.3±8.8 ^d
Everglades41	25.5±3.7 ^c	83.2±2.8 ^{efg}	75.9±11.2 ^c	19.0±2.1 ^{de}	182.1±2.3 ⁱ	729.5±8.1 ^a
ACC3748	41.5±7.3 ^{bc}	113.7±0.6 ^c	107.5±12.7 ^a	26.0±3.7 ^{bc}	241.4±0.5 ^d	672.2±25.7 ^b
ACC4111	44.7±6.7 ^b	113.2±9.4 ^c	108.6±16.2 ^a	30.8±3.6 ^{ab}	263.9±2.8 ^b	694.8±15.6 ^a
ACC4153	39.2±6.4 ^c	91.2±2.3 ^{de}	63.4±8.8 ^{de}	19.5±3.6 ^{de}	217.5±0.0 ^f	517.4±7.3 ^d
ACC4139	36.7±5.8 ^c	96.2±3.3 ^d	86.2±11.4 ^c	22.7±2.7 ^c	164.2±0.0 ^k	571.5±19.2 ^c
ACC4443	20.0±2.5 ^{fg}	82.1±2.7 ^{efg}	70.1±9.8 ^{cd}	18.9±2.4 ^{de}	165.7±0.5 ^k	482.5±1.8 ^d
ACC4751	28.1±4.4 ^{de}	71.5±2.6 ^{ij}	86.9±12.7 ^b	27.4±3.3 ^b	229.0±0.5 ^e	571.0±3.6 ^c
ACC4985	26.1±3.8 ^c	68.4±1.0 ^{jk}	61.7±7.5 ^{de}	15.9±3.3 ^{ef}	142.1±0.3 ⁿ	421.0±1.1 ^g
ACC5014	40.3±5.3 ^{bc}	114.0±13.0 ^c	89.1±14.4 ^b	30.6±4.9 ^{ab}	246.7±4.9 ^c	647.2±8.2 ^b
ACC5072	64.0±10.9 ^a	130.4±2.6 ^b	68.3±9.9 ^d	30.5±8.1 ^{ab}	202.8±4.0 ^g	707.1±17.5 ^a

^zCAI: Chlorogenic acid isomer, ^yCA: Chlorogenic acid, ^xKGRI: Kaempferol glucosyl rhamnoside isomer, ^wKRX: Kaempferol rhamnosyl xyloside, ^vKAPT: Kaemperitrin, The letters above each point indicate a significant difference at the 5% level (Duncan's multiple range tests, n=3).

date, KGRI, KRX, KAPT.

Genetic diversity and relationships

The marker attributes for the SSR primers were summarized as number of allele (NA), number of polymorphic allele (NPA), fraction of polymorphic markers (FPM), GD, PIC, EMR, and MI (Table 5). A total of 229 SSR allele were detected overall, and there were 217 polymorphic allele (94.8%) among the cultivars. The NA for each of the SSR primers ranged from 2 to 8, with a mean of 2.90. The NPA of the SSR primers ranged from 2 to 8, with a mean of 2.75. The average FPM was 0.94 per marker, and the range was from 0.5 to 1. The GD ranged from 0.09 to 0.83, with an average of 0.44, and the PIC values ranged from 0.09 to 0.81, with a mean value of 0.37. The maximum values of

GD and PIC occurred with the KU896464, whereas the minimum values were generated by the DQ068364. The SI values of the SSR primers ranged from 0.02 to 0.73, with a mean of 0.45. The maximum values of SI occurred with KU896449 (Table 5).

A phylogenetic tree was constructed based on the 80 SSR markers in 32 kenaf genotypes (Fig. 2C). The genotypes divided into early flowering, mid-late flowering, and non-flowering groups were grouped into four distinct groups with the exceptions of the two (ACC3748 and ACC5072) genotypes. Group 1 consisted of eighteen early flowering genotypes. Group 2 included both of the non-flowering genotypes (ACC4443 and ACC4751). Group 3 contained eight non-flowering genotypes (Hongma300, Hongma74-3, ACC5014, Everglades41, ACC4985, ACC4135, ACC4111 and ACC4139). Group 4 included mid-late mutant cultivar (Jangdae) and these of original

Fig. 3 Ultra-high performance liquid chromatography (UPLC) chromatogram of kenaf phenolic compounds detected at 280 nm. Peaks 1, 2, 3, 4, and 5 are chlorogenic acid isomer (CAI), chlorogenic acid (CA), kaempferol glucosyl rhamnoside isomer (KGRI), kaempferol rhamnosyl xyloside (KRX), and kaemperitrin (KAPT), respectively. A: C9, B: C20, C: C22, D: ACC4111, and E: ACC5072

Fig. 4 Caliper LabChip GX II patterns following PCR amplification for polymorphic SSR markers in kenaf genotypes. A: KU896464, B: KU896435, C: KU896449. Primer sequences are listed in Table 5

genotype (Jinju).

We explored correlation analysis among the SSR markers, chemical components and morphological characteristics of the kenaf cultivars by applying Q GLM and Q + K MLM

statistical methods (Table 6). A total of 15 significant correlations ($p < 0.05$) were revealed using the Q GLM and Q + K MLM statistical approaches. We discovered four (KU896387, KU896399, KU896441 and KU896449) significant

Table 4 Correlation coefficients between flavonoid content, yield and morphological characteristics of kenaf genotypes ($P < 0.01$)

Trait	R ²
CAI ^z content and flowering date	0.460
CA ^y content and leaf color	0.507
KGRI ^x content and flowering date	0.750
KRX ^w content and flowering date	0.597
KAPT ^u content and flowering date	0.553
TPC ^t content and flowering date	0.638
KGRI and corolla color	0.611
KRX and corolla color	0.496
KAPT and corolla color	0.462
TPC and corolla color	0.516
KGRI and petal color	0.616
KRX and petal color	0.499
KAPT and petal color	0.521
TPC and petal color	0.576
Plant height and flowering date	0.940
DM ^s yield and flowering date	0.985
Seed yield and flowering date	0.901
KGRI and Plant height	0.697
KGRI and DM yield	0.741
KGRI and Seed yield	0.705
KRX and Plant height	0.456
KRX and DM yield	0.557
KRX and Seed yield	0.644
KAPT and Plant height	0.469
KAPT and DM yield	0.526
KAPT and Seed yield	0.597

^zCAI: Chlorogenic acid isomer, ^yCA: Chlorogenic acid, ^xKGRI: Kaempferol glucosyl rhamnoside isomer, ^wKRX: Kaempferol rhamnosyl xyloside, ^uKAPT: Kaempferitrin, ^tTPC: Total phenolic content, ^sDM: Dry matter

correlated with leaf color. The lowest p -value was observed for the KU896449 with leaf color ($P = 2.34E-204$, $R^2 = 0.974$) in Q GLM. Three SSR markers (KU896384, KU896438 and KU896440) had highly significant correlated with petal color. The KU896440 marker had the lowest p -value ($P = 1.36E-07$, $R^2 = 0.206$) by Q MLM. The KU896377 and KU896423 markers had significant correlated with vein color. The KU896426 marker had significant correlated ($P = 0.004$, $R^2 = 0.159$) with KGRI contents. The KU896408 and KU896449 markers had significant correlated with stem color. KU896408 had the lowest p -value ($P = 0.001$, $R^2 = 0.166$) by Q GLM. KU896449 markers had significant (Q GLM: $P = 0.002$, $R^2 = 0.367$ and Q+K MLM: $P = 0.023$, $R^2 = 0.289$) correlated with corolla color.

Discussion

Kenaf is promising potential to exploit and utilize, but

identification of kenaf genotypes and understanding of morphological, chemical and genetic characteristics and relationships is limited, thus significantly hinders their effective utilization (Alexopoulou et al. 2013; Faruq et al. 2013; Jeong et al. 2017). Genetic analyses of the complex quantitative traits involved in morphology and functional compounds are limited (Alexopoulou et al. 2013; Faruq et al. 2013; Zhang et al. 2015). This preliminary study aimed to understand the morphological, chemical and genetic characteristics of these economically important traits, and it is the first exploration of the morphological, chemical and genetic diversity and relationship of kenaf genotype.

Kenaf is considered an important medicinal crop in India and South Africa (Alexopoulou et al. 2013). The leaf has been reported to be anodyne, aperitif, aphrodisiac, fattening, purgative, and stomachic and has long been used as a traditional medicine in Africa and India (Kubmarawa et al., 2009; Alexopoulou et al. 2013; Jin et al., 2013). The leaf contains large amounts of polyphenols, tannins, and other mineral compounds (Kobaisy et al., 2001; Ryu et al., 2017a). Most of the medicinal benefits attributed to kenaf are due to the presence of phenolic compounds (Jin et al. 2013; Zhao et al. 2014; Ryu et al. 2017). The major bio-active compounds in kenaf include the CAI, CA, KGRI, KRX and KAPT. In this study, their content and TPC had a high correlation with flowering date, hypocotyl color corolla color and petal color. KAPT is the main compound present in kenaf leaf. The compound has an acute lowering effect on the blood glucose level of diabetic rats (Jorge et al., 2004). In other studies, KAPT has shown high reactivity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, angiotensin I converting enzyme (ACE) inhibition and also decreased lipid peroxidation (Cazarolli et al. 2006; Jin et al., 2013). The highest level of KAPT content was observed in the C22 genotypes (306.1 mg/100 g) and the lowest level was observed in the C9 genotypes (97.8 mg/100 g). Ryu et al. (2017) reported that the KAPT content ranged from 10 to 178 mg/ 100 g with different solvent extract of kenaf leaves, and that water has the highest KAPT content. The high levels of these compounds observed in the present study, especially for the C22 genotypes, indicate promising commercial potential for kenaf as a source of phenolic compounds.

Analyses of genetic diversity provide for the selection of genotypes in breeding programs and provide useful genetic information (Zhang et al. 2015; Jeong et al., 2017). In this study, high levels of genetic diversity were found for the various kenaf genotypes. PIC and GD detected in this study for kenaf are higher than those previously observed using other marker (SRAP, ISSR, RAPD and AFLP) systems

Table 5 Characteristics of SSR markers for the identification of kenaf genotypes

Genbank ID	Primer sequences (5'-3')	Origin	NA ^z	NPA ^y	FPM ^x	GD ^w	PIC ^v	SI ^u
DQ068360	F CTAGTTTTTGCAGAGGCCAAGT R AGAAGAATTGTTGGCCATGTCT	Genomic SSR Markers	3	3	1.00	0.51	0.46	0.46
DQ068361	F ACTAGTTTTTGCAGAGGCCAAG R GATTACTCATTGCGCATGTCTC		5	5	1.00	0.77	0.68	0.73
DQ068362	F TTACTACCGTTTGAGCGGAGA R CGAATGCCAAGAAAGTTTCAG		3	2	0.67	0.36	0.22	0.33
DQ068364	F GAGGCACTTCAGTGTCTGAGC R CCAATAGGCAGGTTTTTCCTC		2	1	0.50	0.09	0.09	0.02
DQ068366	F TGCCCATTTTTGAGTTTTTAC R TCCTCGAGAAAAAGGATTGTG		2	1	0.50	0.12	0.11	0.12
DQ068373	F ACTAGTTTTTGCAGAGGCCAAG R GAGTGTGTGCATGAAAGGAAA		3	2	0.67	0.54	0.48	0.42
DQ068374	F CACTCCAATCACCATTACAG R CTGATCGAATCCAACCCCTA		2	1	0.50	0.09	0.09	0.02
DQ068376	F CAGTAGCGGACCGTTATTTGA R TTACAGCCTTGGGACTTCAGA		3	3	1.00	0.49	0.43	0.24
DQ068377	F GAATGCAACATTTTTAAATGCAA R GTCTACAAAAGCCAAAGCATAACC		3	3	0.50	0.47	0.42	0.38
KU896377	F CCGAAGCTCCTGCTTTTATC R GTCTCAGATGAAGCCACCAC	EST-SSR Markers	3	3	1.00	0.56	0.50	0.47
KU896380	F GAAGAAACGGGTCATTCTC R GTAGTCGTAGTCATCCTCTGCTC		3	2	0.67	0.53	0.42	0.65
KU896381	F GTGATATCCGAGCACCTTTG R GCGATGATATCAGAACCTCGTC		2	2	1.00	0.50	0.37	0.58
KU896382	F GGTTTTCGGCTCTTGGTT R CTGGACAATTGCGAGAAGAG		3	3	1.00	0.36	0.29	0.48
KU896383	F GCTGAGCAGAGGAGTAGAAGAA R TTCAGCTCAAGCAGTATCCC		2	2	1.00	0.58	0.49	0.60
KU896384	F ATCCTAGTGGATCCCTGAAGTC R GACGATGAGGAGCAGAAAGA		2	2	1.00	0.43	0.34	0.66
KU896385	F GCTGCCATGCTCATGATT R AGCTCACCTCCACTTCTCTAT		2	2	1.00	0.48	0.37	0.63
KU896386	F ACAGCTTTGACTGTCGTCAGTG R AAGTATCTTGTGGGCTGTGG		2	2	1.00	0.45	0.35	0.41
KU896387	F GCCTTCGGAGTAAATGGGT R CACCCAAACATTCTCTCTGG		7	7	1.00	0.32	0.27	0.27
KU896388	F GTTGGTCGTAAAAGCCGAG R AACCCCGTCTTAAACCTCAG		2	2	1.00	0.74	0.72	0.35
KU896389	F GAAACGAAGGGTAGAGTACGGT R GCAGTGTAACAAACAGCCC		3	3	1.00	0.17	0.16	0.44
KU896390	F AGATTGATCTCGTCACCCCT R CCAAACCTGGATCGTAATCCG		2	2	1.00	0.49	0.38	0.55
KU896391	F CAGAAAAGTAGCGGGATGAG R CCACTCGACATTAAACCCAC		2	2	1.00	0.41	0.33	0.54
KU896395	F TACTGGATGAAGGAGTAGCAGC R CTTGATAGGCATCCCTTACCC		2	2	1.00	0.38	0.31	0.36
KU896397	F CCATATAGTTTGGGGGAAGG R CAGTGAGAAGTGAGTGGCTACA		2	2	1.00	0.19	0.18	0.61
KU896399	F AGCCTGTGCTGAAAGCTAGA R GGAGGGAGCATAAGTGAGTTTG		3	3	1.00	0.46	0.35	0.38
KU896400	F CCGACAAGAACAAGTCCA R CAACCCGTGTGCATTGAG		3	3	1.00	0.42	0.35	0.41
KU896404	F GATGGTTTCTCCCAACAACC R CAACGACATCGTCGTCTTC		2	2	1.00	0.52	0.41	0.62

Table 5 Continued

Genbank ID	Primer sequences (5'-3')	Origin	NA ^z	NPA ^y	FPM ^x	GD ^w	PIC ^v	SI ^u
KU896405	F GTCGTCATCATCGTCCAATC R AGATCTCTCTTCACAGTGTCCC		4	4	1.00	0.47	0.36	0.39
KU896406	F CAGTCTGCATCGTCCAATC R AGATCTCTCTTCACAGTGTCCC		3	3	1.00	0.66	0.61	0.33
KU896407	F GCCTTCAGAGAATAGATGTGGG R CAGTTCATCGACTTGGCTTG		3	2	0.67	0.33	0.30	0.49
KU896408	F CTAACACGTCCGGCAACA R GGAGTTCAAGAGGACGTAGTTG		3	2	0.67	0.22	0.19	0.62
KU896409	F CCTCAAGCTCCTCGTAATACAC R GGGTACCAGTGAAGAGAACAAG		2	2	1.00	0.47	0.36	0.49
KU896410	F GTACTTGACGTAGGAAAGGCAG R TTATACGACTCCCCACGGA		3	3	1.00	0.22	0.19	0.42
KU896411	F GTTCCTATGAAGAATCCGGC R ACTTTGAGAGGTTGCAAGGG		2	2	1.00	0.52	0.45	0.56
KU896412	F GTAATCGTTGTTGGCGTTGG R GTCAAACACAAGCTCCAGTCC		7	7	1.00	0.40	0.32	0.31
KU896417	F CCCTCTACCTCTAGGATGATTCTC R ACTAGGTTTCTCTTCAGCGGC		2	2	1.00	0.79	0.76	0.43
KU896418	F GTTCCTTGAGAGAAAGGAGAGG R GTGTTAGTGAGGAGAAGCAAGG		3	3	1.00	0.20	0.18	0.55
KU896419	F GGTAAACTGTTGAAGCGGGT R GCAGAGCATTTCACCAG		2	2	1.00	0.65	0.58	0.42
KU896420	F CCCCTTTTGATCTCTTGC R AGGAGGGAGAGAGAGCTTCA		2	2	1.00	0.26	0.23	0.52
KU896422	F GGCTGCCCTTGCTAATAAGT R ACTCGCTTCTTCATGCTCC		2	2	1.00	0.38	0.30	0.59
KU896423	F GGTGGTTTAAACGAGCACC R GTCTCCCCATTGTTCTCTGA		3	3	1.00	0.43	0.34	0.27
KU896424	F CATCCCGTCTAACACTACATCC R GCACCGAGTATATCCTTCCAC		2	2	1.00	0.17	0.17	0.66
KU896426	F GGAGTCGTATAATGGGGTGA R CTCCCTCTCGAAAATACGTAGC		3	3	1.00	0.50	0.37	0.44
KU896427	F GGTATGGCAGACGAGATGTT R GTGTTAGTAGGCACTGGTGAAG		2	2	1.00	0.44	0.40	0.39
KU896428	F GCTCCTGCACTGTTTGTGT R CTAGGCTTATGTGTGGACCG		2	2	1.00	0.22	0.19	0.45
KU896429	F GGAGTGTCTTGTAATAGCCAC R CTCCAACCTCCCATTGTTT		4	4	1.00	0.30	0.26	0.39
KU896430	F GATCCGAAGGTAAATGGGTC R CAGACACCTTTAGCCCCAC		2	2	1.00	0.64	0.60	0.40
KU896431	F AATCCAGGGAAGCAGCTC R GCATATCTCTGAAGTGTCTCCG		4	4	1.00	0.22	0.20	0.39
KU896434	F CACTAAGAGCCCAGAAAGAAGC R GAGACTCTTGTGGAGTTTCTGC		2	2	1.00	0.61	0.55	0.27
KU896435	F GTAGTCACCGCCGTCACAATAG R CTATTCTGGCTCTCCCAACA		2	2	1.00	0.12	0.11	0.44
KU896436	F CGGCTGTTACTCCATCAAAG R GGTCGTCTTACAATGGTTCC		3	3	1.00	0.22	0.19	0.44
KU896438	F ATTCAGAAACCGATGCCC R GGAATGTCACTGGTCCGAG		3	3	1.00	0.49	0.41	0.59
KU896439	F AGCTCCGGGGATAAGTTAAG R GCCTTCTCTCCTTGACCAGTA		3	3	1.00	0.61	0.53	0.42
KU896440	F GGCACAATAGAAGAGGCAGACT R CTTCGAATTTACGCGTCG		3	3	1.00	0.48	0.39	0.43

Table 5 Continued

Genbank ID	Primer sequences (5'-3')	Origin	NA ^z	NPA ^y	FPM ^x	GD ^w	PIC ^v	SI ^u
KU896441	F GAGATGTTTGATGCTCCAGG R TCACGAAACCAAAGCAGC		2	2	1.00	0.39	0.33	0.46
KU896442	F CACAGTTTCACGAGGCTAACTC R GAGAAGAGCTTCCAACCAGG		8	8	1.00	0.28	0.24	0.22
KU896443	F GTACACAAAGTGCAACCTCTCC R TTCTCCCCTAATTCTCTCACC		2	2	1.00	0.73	0.69	0.60
KU896444	F CGTTCAACATCTCCAGAACC R GAGTACGAGTTCAGCTGTAGCA		2	2	1.00	0.43	0.34	0.61
KU896445	F AACGTCGGCTGCAACTTT R GGGCTTGAAAGTGTCGAGAA		2	2	1.00	0.48	0.36	0.51
KU896447	F GGTGATCAGCTCGAGTCC R AAATCCACCTCATCTCCAGC		3	3	1.00	0.50	0.37	0.46
KU896449	F CTCCTCAATCTAAGCCGTCC R ACGATCACGACTTGCTCTTC		3	2	0.67	0.53	0.44	0.66
KU896452	F TTCACTTCAGCAGACTTCCC R GATGCTCTGGGTGTGTAGA		3	3	1.00	0.48	0.37	0.38
KU896454	F GCTTGGCTTCAACTCATCTC R CGGCGGCTTTTATAAGGA		4	4	1.00	0.28	0.25	0.37
KU896455	F GGAGTGTCTGTAAATAGCCAC R CTCCAACCTCCCATTGTTC		6	6	1.00	0.57	0.52	0.30
KU896456	F GAAACCGTGTGGTCTTGTC R AAAGGCCCGATCCAAATC		3	2	0.67	0.69	0.66	0.63
KU896457	F GACCACCTCGAGAATAAGCA R CTCCCAGGTAACGTCGAAT		2	2	1.00	0.50	0.37	0.53
KU896458	F CTCTGGAGAAGCTAAGGAGTGA R CCATGTTCTCAAACCCTTCC		2	2	1.00	0.40	0.32	0.54
KU896459	F ACAGCGTGTGGAGGTTTATA R AGCAGCCACCGTCTAAAAG		2	2	1.00	0.40	0.32	0.51
KU896461	F GGCAGGATATTCGACGGT R GGAGATGGTCTCTGTTAAGG		3	3	1.00	0.36	0.29	0.49
KU896462	F CAAGGCTTAGGTCGTAGGTATC R AAGAGAAGCCAAGATCAGCC		4	3	0.75	0.62	0.54	0.45
KU896463	F CCATGGTCATATTGCTCTCC R TGTACTACTCTCTCTGCTCTCC		7	7	1.00	0.52	0.41	0.28
KU896464	F CCAGGAATCTATTGTCGGG R CAATAATTCAGCCCTCCCTC		2	2	1.00	0.83	0.81	0.56
KU896466	F GCTGTTTCTTAACAGGAGCAGG R GGTGTAGCTCAGGCTGTTGTA		4	4	1.00	0.40	0.32	0.35
KU896468	F CGGGTCTTACGTTCCCTAGTA R CCAGCTCCATTGATCTTTCC		3	3	1.00	0.33	0.31	0.48
KU896471	F GGATCGAAACAACCCAGTC R TCAACCAAACCAACTCC		3	2	0.67	0.59	0.52	0.60
KU896473	F ATCGCAGGATCTCTCCAAG R GATGGATTACTTCCCCTAGGAC		2	2	1.00	0.50	0.39	0.59
KU896476	F CTCTCTTCTCTCAAACACCC R GGGTAAGAAAAGGGCAGACA		4	4	1.00	0.46	0.35	0.34
KU896477	F CTCATCTCTGCACTTCCAT R GGCAATTGCATCGTCAAG		2	2	1.00	0.38	0.33	0.46
KU896478	F GTCTTGGGAGTGGCTTTTGT R CTCATCATTTACCTCCGACG		2	2	1.00	0.28	0.24	0.58
Mean			2.90	2.75	0.94	0.44	0.37	0.45

NA: Number of alleles, ^yNPA: Number of polymorphic alleles, ^xFPM: Fraction of polymorphic marker, ^wGD: Gene diversity, ^vPIC: Polymorphism information content, ^uSI: Shannon's Information index.

Table 6 Association analyses between SSR markers and morphological characteristics and phenolic compound contents of the kenaf genotypes

Marker	Trait	Q GML <i>P</i> -value	R ²	Q+K MLM <i>P</i> -value	R ²
KU896377	Vein color	*	0.266	*	0.248
KU896382	Total polyphenol	*	0.123	*	0.131
KU896384	Petal color	***	0.142	*	0.363
KU896387	Leaf color	***	0.303	*	0.247
KU896399	Leaf color	*	0.203	*	0.231
KU896408	Stem color	*	0.166	*	0.187
KU896423	Vein color	*	0.258	*	0.233
KU896426	KGRI	**	0.159	*	0.160
KU896438	Petal color	***	0.140	*	0.309
KU896440	Petal color	***	0.206	*	0.246
KU896441	Leaf color	***	0.973	**	0.999
KU896447	Leaf shape	**	0.235	*	0.181
KU896449	Leaf color	***	0.974	***	0.999
KU896449	Corolla color	***	0.172	*	0.289
KU896449	Stem color	*	0.219	*	0.248

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(Cheng et al. 2004; Chen et al. 2011; Zhang et al. 2013). In additions, these results are higher than the findings of Li et al. (2016) in 28 kenaf cultivars using 72 EST-SSR markers. Especially, the maximum values of GD and PIC occurred with the KU896464 and the maximum values of SI occurred with KU896449. This suggests that KU896464 and KU896449 markers could be used to assess genetic diversity in genotypes.

The correlation analysis showed flowering time and DM yield, seed yield and phenolic compounds were significantly correlated. Also, comparison of three phylogenetic tree based on morphological and chemical characteristics with those based on SSR data showed that the groups formed by the six early flowering genotypes (C9, C11, C14, C15, C17, C19) grouped similarly in SSR phylogenetic tree. Ultimately, early flowering, mid-late flowering and non-flowering groups were clearly divided by genotypes in SSR phylogenetic tree. Similarly, Jeong et al. (2017) reported that phylogenetic and population structure showed that the 45 accessions could be clearly divided into three groups based on different days to flowering by EST-SSR markers through de novo RNA sequencing. The flowering period has been reported to be an indication of sensitivity of kenaf genotypes to photoperiod, later flowering genotypes being photo-insensitive (Webber et al. 2002). Korean Kenaf cultivars are divided into three maturation groups depending on flowering date; early-flowering, mid-late flowering, and late flowering (Kang et al., 2016). Early-flowering cultivars bloom in 70 ~ 80 day after sowing. Such varieties allow seed harvested in Korea, but the brief vegetative growth period produces shorter plants of lower biomass (Webber and Bledsoe, 2002; Kang et al., 2016). Late-flowering

cultivar grows vegetative for 130 ~ 140 days and yield significantly higher biomass. However, late flowering increases the risk of seed shattering (Kang et al., 2016). To achieve profitability, the selection of the best cultivar for each country is important. The breeding of kenaf cultivars for yield and economic important with adaptation to local conditions has been conducted actively using these genetic resources (Alexopoulou et al., 2013; Kang et al., 2016). Breeding selection of kenaf starts mostly from the introduction of new cultivars, and the breeding selection of new cultivars is the most effective measure for increasing yield and improving functional quality (Ryu et al., 2016; Jeong et al., 2017). The Jangdae and Jeokbong cultivars were derived by gamma ray (300 Gy) treatment. The mid-late cultivar Jangdae, which affords both high biomass and high seed yield, has been registered in Korea (Kang et al., 2016). The Jeokbong cultivar has distinctive morphological characteristics such as dark purple color. The low levels of phenolic compounds observed in the present study for the Jeokbong cultivar, but their antioxidant and antioxidant and ACE inhibition activity are approximately 4 ~ 5 times higher than other cultivars caused by anthocyanin (Ryu et al., 2017b). Based on the flowering time, the morphological and chemical phylogenetic tree revealed an unclear pattern of division between mutant cultivars (Jangdae, Baekma and Jeokbong) and these original genotypes (Jinju and C14), while the genetic phylogenetic tree showed that the mutant cultivars grouped with the those original genotypes (Jinju and C14). Thus, these results could be used for the selection of kenaf cultivars with improved yield and functional compounds. Our results of morphological, chemical

and genetic phylogenetic trees were not related with the origin. In previous studies on the origin, many studies agree that kenaf originated from Africa (Dempsey 1975; Alexopoulou et al. 2013). However, the knowledge of how kenaf was introduced in Asia is limited but it is known that it came from Africa (Dempsey 1975; Alexopoulou et al. 2013; Zhang et al. 2013). In addition, it is likely that cluster analysis using country of origin was not able to differentiate among all cultivars because of the limited number of accessions. The SSR phylogenetic tree clearly showed that the genotypes divided into flowering time, but the genomic and EST SSR markers not significantly correlated with flowering time. Marker assisted selection has the potential to improve the efficiency of plant breeding because of its increased accuracy and reliability. However, with this large average distance, greater saturation would be needed for practical application especially for marker assisted selection (Varshney et al. 2005; Varshney and Tuberosa, 2007; Zhang et al. 2011). To the best of our knowledge, this is the first report on the correlation of molecular markers with phenolic compounds, morphology and agronomic traits in kenaf. These 15 SSR markers could be used for selection of kenaf cultivars with improved morphological characteristics and functional compounds (total polyphenol and KGRI). The mapping of genes controlling agronomic traits and functional compounds coupled with the widespread availability of easy to use simple sequence repeat (SSR) markers (Varshney et al. 2005; Varshney and Tuberosa, 2007). These results could be used for the selection of kenaf cultivars with improved yield and may be a good candidate for pharmaceutical products.

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