여교잡을 통한 케나프 바이오매스 우수 계통 선발

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Development of Kenaf (*Hibiscus Cannabinus* L.) Pedigrees with Superior Characteristics through Backcross

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Abstract Multipurpose utilization, including as rope, pulp, and filtration media, is what makes kenaf (*Hibiscus cannabinus* L.) special. This experiment was conducted to select elite pedigrees with high biomass production through backcrosses. The agronomic performance, fuel characteristics, and histological analysis of the elite lines were investigated at the BC₁F₂ generation in comparison with the Control. Significant differences were observed in 12 evaluations of variety and pedigrees. Five plants, including the two Controls (Jangdae and Hongma300) and three pedigrees, showed a large difference in the number of flowering days from July 5 to September 30. The plant height of BC100-10 (447 cm) was the highest among the genotypes. Regarding branch number, BC100-15 showed the best performance with 29 branches. Stem dry weight was found to be the lowest in Jangdae at 792 g, whereas BC100-17 showed the highest dry weight. Hongma did not set seeds owing to late flowering. However, with 34 g/plant on average, three elite lines had more seeds than Jangdae (16 g/plant), which are vulnerable to decay owing to viviparous germination. In the fuel characteristics analysis, the heating value of the three elite lines ranged from 4,510 to 4,930 kcal/kg, which was much higher than that of the two Controls (4,320 kcal/kg). The ash content analysis indicated the lowest value at 3.2% for the line BC100-10, which was similar to Korea's 3rd-grade. Based on the histological analysis of the stem, the pedigrees were close to Hongma. Taken together, these three elite lines would be useful as genetic resources for high biomass production.

Keywords kenaf, pedigree, fuel characteristics, histological analysis

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Introduction

Kenaf (*Hibiscus cannabinus* L.), which belongs to the family of Malvaceae, is an annual C3 and a common warm season fiber plant native to India and Africa (Yazan et al. 2011). It is one of the most important fibre crops in the world. It has been cultivated and used twine, rope, gunny bag, sackcloth, pulping and paper making, oil absorption, potting media, board making, filtration media and animal feed (Dempsey 1975, Sellers & Reichert 1999, Cheng 2001, Charles 2002). Also, the seeds are good source of low cholesterol vegetable oil and for biodiesel production (Webber & Bledsoe 1993). Although kenaf is a tropical plant, its cultivars are now well adapted to a wide geographical and climatic range (Danalatos

& Archontoulis 2010). Its height reaches 4-6 m in about 4-5 months and a yield is 6-10 tonnes of dry mass per acre each year, which is 3-5 times greater than the yield for the southern pine tree taking 7-40 years to be used (LeMahieu et al. 2003). The 'green tag' is further associated with kenaf because of its promising growth, and scavenges extensive amounts of Carbon dioxide (CO₂) from the atmosphere. It is the most abundant gas which could give severe effects to the global warming. So, kenaf is the best suitable resource to go green.

The Renewable Energy Portfolio Standard (RPS) has been operated in 44 countries including USA, Japan, and so forth. Since 2012, it has been implemented in Korea. The policy's aim is to increase expansion of a new renewable energy to decrease the CO₂ of an atmosphere. So, a lot of wood pellet

have been imported in Korea to be used in a thermoelectric power plant from 2013 year. To my surprise, its amount of income was 540 billion Won in 2018 (Kim et al. 2020). More seriously, the harmful insects such as red imported fire ant (*Solenopsis invicta*) and Fire Blight (*Erwinia amylovora*) have been coming in with the imported pellets, causing a severe social problem.

Kenaf is commercially cultivated in more than 20 countries, particularly in India, China, Thailand and Vietnam (Olasoji et al. 2014). This plant was introduced in Korea in the 60's. However, it has limited use in the country. In recent years, its value in Korea has been increasing as it has been used as forage, and for biomass and fuel production. Researches on kenaf have been done by the different research institutions in Korea since 2003 which focus on the development of new varieties, agronomic practices, and downstream application development studies such as animal feed (Kang et al. 2018, Lee et al. 2018). To successfully cultivate kenaf in Korea, the development of its new varieties producing high biomass is essential. Unfortunately, the yield of an early maturing kenaf variety in Korea is still very low. The highest yield achieved for early maturing varieties were 14.8 and 16.1 ton per hectare only in Jeokbong and Jangdae, respectively, thus becoming a major limitation for commercial use (Ryu et al. 2018). Of all the available early maturing kenaf varieties, only a few were suitable to be planted in Korean climate. The late flowering kenaf plant variety has been identified as the most essential trait required for growing in Korea to increase biomass production. However, this variety was not able to bear seeds in Korean climate. Thus, in order to overcome the problem

on the lack of varieties, the development of a new kenaf variety with selected target traits is imperative.

Thus, the main objective of this study was to generate new kenaf varieties with high biomass through backcross breeding. Also, it aimed to describe pedigrees in regards to their morphological, genetic and histological traits in comparison to the Control varieties, Jangdae and Hongma, in BC₁F₂ generation.

Materials and Methods

Location, experimental design, treatments, and agronomic practices

A field study was conducted for 4 years, from 2017 to 2020, at Iksan, Korea (35.9 N, 127 W) on a fine sandy loam, 1~2% slope. The field was cleaned and treated with 3 kg Pendimethalin herbicide 10a⁻¹. Before planting, fertilizer was applied at a rate of 15 kg 10a⁻¹ N, 10 kg 10a⁻¹ P and 10 kg a⁻¹ K. A field cultivator was used to incorporate the fertilizer and to prepare the seedbed for planting. Planting was done at 80 × 40 cm distance on 1st May. The primary weeds in the field were tumble pigweed (*Amaranthus albus* L.), purslane (*Portulaca oleracea*, Purslane), and removed throughout the growing season by herbicide and handweeding.

Climate conditions during the experimental period

During the experimental period, from 2017 to 2020, the average minimum temperature in the area was 17.7°C and average maximum temperature was 26.7°C, with a total of 580~1,525 mm rainfall (Table 1). The optimum temperature for kenaf to grow ranges from 20-27°C (Koester et al. 2014)

Table 1. Climatic conditions of field during the experimental period, from May 2017 to October 2020.

Region	Temperature (°C)			Rainfall (mm)
	Av. minimum	Av. maximum	Av.	
2017	17.3	25.3	22.5	580
2018	17.9	27.7	22.3	1,022
2019	17.9	27.3	22.1	696
2020	17.6	26.6	21.4	1,525
Av.	17.7	26.7	22.1	956

*Av. : Average

and rainfall of 500-1,200 mm (Banerjee et al. 1988, Bañuelos et al. 2002). Thus, the climatic conditions in Iksan City is very suitable to raise kenaf.

Pedigrees preparation, screening of elite lines and fuel characteristics evaluation

A selection step of the elite lines was done (Fig. 1). Two varieties, Jangdae and Hongma300, were used as parental lines for crossing. F_1 lines were established in 2017 using Jangdae as female and Hongma300 as male resource. In 2018, backcross was implemented with Jangdae used as recurrent parent and harvested BC_1F_1 seeds. The seeds of previous year were planted, harvested BC_1F_2 seeds, and since then assigned numbers for 22 lines in 2019. 20 seedlings per each pedigree of BC_1F_2 generation were grown to maturity in an upland field in 2020. The elite lines showing normal seed fertility were investigated on individual plants. The stem color, leaf shape, 1st flowering date, capsule shape, plant height, stem diameter of 15 cm above soil surface, branch number, dry weight, and seed weight were recorded for three elite lines and compared to the Control plants (Jangdae and Hongma300) evaluated in 10 replications. All genotypes were evaluated for the anthracnose incidence, using a five-point scale method (0 point = Disease free, 1 point = 1~20% incidence, 2 point = 20.1~30% incidence, 3 point = 30.1~40% incidence, 4 = point over 40.1% incidence). The fuel characteristics was evaluated from KTR (Korea Testing

& Research Institute), which is a national accredited agency, according to Ministry of Environment Notice 2020-219 in 2020 (Seo et al. 2015).

Scanning electron microscope (SEM) image

The stems were air-dried for 48 hours at 30°C. Each stem was then fastened using a nipper before cutting vertically to avoid mechanical injury for the vertical section filming. The specimens were air-dried for 20 hours at 80°C to shoot, then placed on an aluminum stub, and plated with gold using a gold ion sputtering device (Jeol, JFC-1100E, Fine Coat, Tokyo, Japan) at 10 mA for 400 seconds. A mutant stem plated with gold was observed using a scanning electron microscope (Jeol, JSM-5410LV, Tokyo, Japan) at 15 kV condition (Bednorz et al. 2008).

Statistics analysis

Results were analyzed for analysis of variance (ANOVA) using SAS Enterprise Guide 4.2 (Statistical Analysis System, 2009, SAS Institute Inc., Cary, NC, USA). Also, comparison of means was done at 5% level of significance using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Characteristics of donors

The elite pedigrees were developed from a cross between 'Jangdae' × 'Hongma300'. The female parent released from KAERI (Korea Atomic Energy Research Institute) is of Korean origin. The cultivar divided into mid-late flowering group has been flowering for three months from early-July to late-September, when planted on May 1. As a result, it can be harvested up to 16.1 ton per hectare at 100 days after seeding that is a relatively low yield (Kang et al. 2016). However, the male parent introduced from China has been designated as late-flowering, since the first flower blossoms in late-September. In the end, it has a high biomass production of 17.9 ton per hectare at the same harvest time with Jangdae (Kang et al. 2016). Accordingly, growers have been preferring Hongma300 to Jangdae for an aerial-part biomass.

Fig. 1. Selection steps of kenaf elite lines.

Selection intensity

To develop new superior genotypes compared to its parent, backcross method has been used in a lot of crops (Ali et al. 2006, Behmaram et al. 2014, Noori et al. 2016). Screening and selection of desired lines were the most critical step in early generation. Assessment of important morphological traits in BC₁F₂ progeny is common. 440 BC₁F₂ populations were planted and primarily selected for the flowering date, anthracnose and biomass amount of aerial part through at least twenty observations. Finally, the progenies showing transgressive performance of the target traits over the parental lines were obtained. The selection intensity showed 0.68% with three elite lines picked out. This suggests the presence of gene for improved biomass amount in the donors, which in some cases were not expressed in the donor phenotype. Ali et al. (2006) reported a result in agreement with ours.

Plant characteristics: stem color, leaf shape, flowering date, capsule shape, anthracnose

Through single-crossing, it was impossible to select an elite line satisfying a flowering date we want. So, backcross program was implemented. Results on stem color, leaf shape, 1st flowering date, and capsule shape of the Controls and pedigrees were presented (Table 2). The stem color among the three pedigrees was significantly different from Hongma300, being similar to Jangdae in green. The leaf shape of all the genotypes was palmate. The 3 lines (BC100-10, BC100-15, BC100-17) came into blossom at a period of August 12 and 31, which were a big difference in the number of flowering days of Jangdae on July 5 and Hongma300 on September 30. The capsule shape of all genotypes was same as ovoid. The incidence of anthracnose

for the 3 pedigrees was classified as 1 point with Hongma300 compared to Jangdae of 3 point in the range of 30.1~40%. Anthracnose is perhaps the most serious potential disease problem. So, the anthracnose-resistant varieties were bred in a heavily disease field (Li & Huang 2013). Similarly, the useful lines through the backcross program was obtained by other studies for rice and kenaf (Ali et al. 2006, Behmaram1 2014).

Plant characteristics: plant height, diameter, branch number, dry and seed weight

Fig. 2 shows the varying plant height by the genotypes. The range of plant height was the highest in the line BC100-10 with 447 cm among the genotypes. However, no statistical difference was noted when comparing the two Controls and the average of the three genotypes be marked with the slashes. No statistical analysis for the three mutants was carried out because there was only one lines in BC₁F₂ generation. The

Fig. 2. Difference of plant height for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100-15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

Table 2. The Different characteristics among BC₁F₂ generation kenaf elite lines from vegetative to reproductive growth period.

Genotypes	Stem color	Leaf shape	Flowering period	Capsule shape	Anthracnose ^z
Hongma300	Light red	Palmate	After Sep. 30	Ovoid	1
Jangdae	Green	Palmate	Jy. 5~Sep. 30	Ovoid	3
BC100-10	Green	Palmate	Aug. 12~Sep. 30	Ovoid	1
BC100-15	Green	Palmate	Aug. 16~Sep. 30	Ovoid	1
BC100-17	Green	Palmate	Aug. 31~Sep. 30	Ovoid	1

^z0 (Disease free), 1 (Rate of 1~20%), 2 (Rate of 20.1~30%), 3 (Rate of 30.1~40%), 4 (Over 40.1%)

result was same to another study which showed that the kenaf, sweet sorghum and okra pedigrees of backcross breeding program gave a significantly morphological difference in comparison to Controls (Behmaram1 2014, Bunphan et al. 2014, Chavan et al. 2019).

Fig. 3 shows the results on the diameter of the test plants. Individual comparison of the two Controls and the three lines indicated only one line (BC100-17) having a wide gap with the Control and other lines. Behmaram1 et al. (2014) revealed that a significant difference was not observed in the diameter between the two Controls and BC₁F₂ populations. One distinctive feature showing a big difference between our finding and previous author's result is a difference of parental lines. We used the mid-late and late flowering varieties with a difference of 85 days in flowering. On the other hand, the early flowering varieties were utilized by Behmaram1 et al. (2014), showing the maximum difference of 20 days in flowering.

Result from Fig. 4 indicated that the range of branch number of the pedigrees was different with the two Controls. The means of branch number for the Controls were from 10 (HM) to 13 (JD), while the pedigrees ranged from 15 to 29. There was a highly significant genotypic variation among the genotypes. Based on the results, line BC100-15 had the most number of branches that is, 29, followed by line BC100-17 with 19, and the lowest in line BC100-10 with 15. This conformed

Fig. 3. Difference of stem diameter for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100 15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

that a high level of genetic diversity for this trait was generated through crossing. It was indicated that this trait would respond very well to continuous selection (Panse 1957). Akinrotimi & Okocha (2018) revealed that the wide variation in the agro-morphological characteristics of the crop proves there is a wide genetic diversity among the genotypes. Also, number of branches per plant exhibited higher percentage of genetic advance (Malek et al. 2014).

As to a leaf dry weight range per plant, the pedigrees showed approximately a middle value between HM and JD (Fig. 5). The means of dry weight for the HM and JD were 317 and 153 g/plant respectively, while pedigrees ranged from 227 to

Fig. 4. Difference of branch numbers for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100 15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

Fig. 5. Difference of leaf dry weight for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100 15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

262 g/plant. Notably, the highest leaf dry weight among pedigrees is line BC100-10, followed by line BC100-15, and the lowest was line BC100-17. There was something unusual about the relationship between the branch and leaf for line BC100-15. This line had the largest number of branches of 29 (Fig. 4), but the weight of the leaves was similar to that of other pedigrees (Fig. 5). The genotype's branches mainly occurred on the upper part of plant from early September, producing short branches with fewer leaves. It was thought that because of such growth property, the branch number increase didn't lead to the leaf dry weight increase. So, we are going to precisely investigate whether this phenomenon will be emerged in the next generation. Some authors likewise noted significant difference on leaf dry weight when compared to the genotypes (Hossain et al. 2011, Noori et al. 2016). Previous two authors reported that an increase of leaf dry weight didn't raise the increase in stem dry weight.

The stem dry matter accumulation at harvest differed significantly among the genotypes (Fig. 6). Of the pedigrees, the highest dry matter of stem (1,519 g) was produced in line BC100-17 which was followed by the stem dry matter (1,219 g) of line BC100-15 and BC100-10 had the lowest (1,072 g) stem dry matter. Overall, the breeding lines have more stem dry weight than the two Controls. These findings are in agreement with the results of Al-Amier et al. 2008, Joshi et al. 2016 who reported the segregating lines with the highest stem dry

Fig. 6. Difference of stem dry weight for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100 15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

matter in kenaf and pennyroyal. And, other authors showed that the stem dry weight of the BC₁F₂ genotypes was a big difference depending on the recurrent line (Behmaram1 et al. 2014, Noori et al. 2016). Also, high heterosis for yields related traits was reported on kenaf (Liu 2005).

Fig. 7 shows the analysis of seed weight per plant between the Controls and pedigrees. The Control (HM) did flower on September 30. So, the seeds weren't developed enough to germinate due to a low temperature. Individual comparison of the Control (JD) and the pedigrees showed that all lines had significant variation. The means of seed weight for the Control (JD) were 16 g/plant, while pedigrees ranged from 26 to 45 g/plant. Among the pedigrees, the line BC100-10 with weight of 45 g had the highest value and the line BC100-17 with 26 g weight had the lowest value. Results indicated that most of the new pedigrees showed better performance than the Control (JD) with the significant variations in weight of seeds per plant, with these results confirming other studies (Banerjee et al. 1988, Osman et al. 2011). The seed yield range of three lines is considered to be 200-375 kg/1,000 m². Literature reported a seed yield in a range of 100-150 kg/1,000 m² in US for 'SF-459' and Mexico for 'Everglades 41', however, a seed yield for 20 varieties (2QC etc.) in Nigeria is lower than 100 kg/1,000 m² (Olasoji et al. 2014). Kenaf has an indefinite inflorescence trait. The viviparity occurred at the seeds that have been bloomed early due to much rainfall in

Fig. 7. Difference of seed weight for HM, JD (Control) and BC₁F₂ three pedigrees in 170 days after planting. Result of two Controls is shown as mean $\pm$ standard deviation, obtained from ten replications. Av. (BC) indicates the mean of the three pedigrees (BC-100-10, BC-100 15, and BC-100-17). A statistical analysis for the three pedigrees cannot be carried out because each line is only one in BC₁F₂ generation. HM and JD indicate Hongma300 and Jangdae, respectively.

Korea, lowering a germination. So, the seed yields in kenaf are a top-priority breeding goal to come over the problem. Three lines with high seed yield and biomass yield potential were identified for the promising performance in Korea. These genotypes showed promising results as dual purpose kenaf lines; seed and biomass production.

Fuel characteristics

The pellets appearance showed a bright color with a high gloss and there were no bad features of making pellets. So, we concluded that a pellet's formability was very good in appearance. Fuel characteristics was done with those pellets (Table 3). As for the heating value, the three lines ranged from 4,510 to 4,930 kcal/kg which was much higher than two Control varieties with 4,320 kcal/kg. It is excellent enough to satisfy the quality standards of Korea's 1st-grade. This result

demonstrates that it is higher than that of reed which belongs to an herbaceous plant (Kim & Han 2016). To my surprise, the results of the elite lines was consistent with previous studies (Kim & Han 2016, Kim et al. 2020) which used wood such as larch and acacia. The ash content analysis produced the line BC100-10 showed the lowest value with 3.2% which was approximate to the Korea's 3rd-grade. Based on the result, the kenaf's ash quantity was a lot lower than that of reed (Kim and Han 2016). So, the kenaf has an advantage to use as a raw material for biomass pellets.

Comparison of scanning electron microscope (SEM)

Histological difference was detected using scanning electron microscope (SEM) image (Fig. 8). The SEM image of stem tissue between Jangdae and Hongma300 differed significantly. The lumens (L) in core fiber of all elite lines were similar

Table 3. Fuel characteristics of kenaf pellets.

Items	Hongma300	Jangdae	BC100-10	BC100-15	BC100-17
Heating value (kcal/kg)	4,320±75 ^a	4,300±65 ^a	4,510±90 ^b	4,680±70 ^c	4,930±110 ^d
Ash (%)	3.4±0.1 ^{ab}	3.3±0.05 ^b	3.2±0.15 ^b	3.6±0.15 ^a	3.4±0.1 ^{ab}

1st grade of heating value: $\geq 4,300$, 3rd grade of ash content: ≤ 3.0

Means with the same letter are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

Fig. 8. Histological analysis of Jangdae (JD), Hongma300 (HM) and line BC100-10, BC100-15 and BC100-17 by scanning electron microscope (SEM) image. The lumen (L) in the three lines was similar to HM being used as donor. Bar = 100 μ m.

to the Hongma300, which was smaller than the Jangdae (Fig. 8). It was thought that this dense connective tissue can increase the density of the pellet. High-density materials showed a higher mass in relation to volume and thereby they have a higher combustion yield. It was reported that high density affected the fuel value index of biomass for energy generation (Hersztek et al. 2019). It has been the first report to reveal a tissue difference between the Control and backcross pedigree in kenaf.

From all the experimental results, the selection for isolating the elite pedigrees was highly probable for high biomass production. Finally, the selection in BC₁F₂ generation would increase the probability of identifying superior lines, while the three pedigrees can be useful as a resource for high biomass production in Korea.

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