Character Association and Selection of Breeding Line Based on Morphophysiological Characteristics and Tensile Strength in *Hibiscus cannabinus* L.

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This study aimed to access different desirable characteristics of nine *Hibiscus cannabinus* L. accessions based on morphophysiological characteristics and fiber tensile strength for an effective selection of *H. cannabinus* plant improvement. Four China accessions (FH952, T15, T17, and T19), four Bangladesh accessions (HC2, HC95, V4202, and V4383), and V36 (control) accession were examined in a four-month cultivation period. The experimental design was arranged using randomized complete block design with three replications. Stem diameter was found to be significantly related ($p \leq 0.05$) with all morphological and yield characteristics except for leaf dry weight and growth efficiency. Bigger stem diameter was an indicator of fiber yield in attempts to apply crossing and selection to improve performance. Photosynthesis rate also was found to be significantly related ($p \leq 0.05$) with stomatal conductance, transpiration rate, instantaneous water use efficiency, and carboxylation efficiency. High photosynthesis rate could be an indicator to interpret the pattern of genetic variation of plant assimilation rate and its relation with environmental and agronomic factors. The fiber tensile modulus, however, was found to be inversely correlated with fiber diameter. The present study suggests the selection of control, V4383, HC2, and FH952 accessions for a breeding line as they possess high fiber yield, fiber strength, and photosynthetic efficiency.

Keywords: Kenaf; Fiber; Morphology; Physiology; Tensile strength

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INTRODUCTION

Kenaf, which is scientifically named *Hibiscus cannabinus* L., is an agro-based lignocellulosic that is grown specifically for its fiber. Various annual crops such as cotton (*Gossypium hirsutum*), hemp (*Cannabis sativa*), jute (*Corchorus capsularis*), kapok (*Ceiba petandra*), and coconut (*Cocos nucifera*) are known to produce natural fibers. *H. cannabinus* however has advantages of harvesting operation and cost-effectiveness because the fiber is obtained from its vegetative plant part (stem) instead of reproductive plant part, and the fiber yields are greater than those of the above mentioned crops (Tahery 2011). Being comprised of 65% woody inner core and 35% fibrous outer bast makes *H. cannabinus* a hard fiber plant with an excellent source of cellulose fiber (Tahir *et al*. 2015). The yield and fiber quality such as fiber diameter, fiber length, and fiber strength, however, differ greatly among cultivars; these traits can be affected by genetic inheritance and manipulation, environmental factors, and also by their interactions (H'ng *et al*. 2009; Abdul Khalil and Suraya 2011; Faruq *et al*. 2013).

The yield, for example, is firmly related to plant growth performance and their physiological attribute such as photosynthesis rate (Evans 2013). The products of photosynthesis are utilized in the development and differentiation of new cells as well as supporting the existing tissue of the plant. The greater photosynthetic rate and high specific activities of RuBP carboxylase possess higher biomass production as well as fiber yield. The superior photosynthetic rates and high biomass yield are also related to the amount and the efficiency of carboxylation capacities in leaf that considered as regulating the photosynthetic capacity in C3 plants (Acquaah 2007). However, Reddy and Das (1986) suggest that losses of available energy to support photosynthesis are due to physical properties of leaves and more fundamental energy considerations for successful conversion and storage of sunlight as chemical energy in the photosynthetic process. To assess the efficiency of the photosynthesis process in a plant, knowledge of total plant biomass production must be acquired. Morphological and physiological attributes also help to better understand the plant plasticity and adaptive mechanism (Abdul-Hamid *et al.* 2009).

Furthermore, fiber strength and micronaire are associated with cellulose deposition within fiber and the developmental process, which are related to fiber secondary wall characteristics (Wang *et al.* 2009). The manipulation of the relationship between the photosynthetic assimilate source and the reproductive sink also would affect fiber strength and micronaire formation (Chen *et al.* 2017). Lokhande and Reddy (2014) reported that a decrease in leaf water potential resulted in decreases in fiber properties such as fiber length, strength, and uniformity, but an increase in fiber micronaire. However, the alternation in the available assimilate supply to the developing bolls affects fiber quality less directly than the yield (Pettigrew 2001). Therefore, better understanding of the responses of leaf photosynthesis is crucial in order to enhance the yield and improve fiber quality (Long *et al.* 2006; Khan *et al*. 2019). In this study, the authors investigated morphophysiological quantitative characteristics and tensile strength of nine *H. cannabinus* accessions as a breeding line selection for a future *H. cannabinus* breeding program.

EXPERIMENTAL

Materials

Nine *H. cannabinus* accessions were selected in this study from three different origins (Table 1). The accessions were selected based on the availability in the previous study of the accessions in Malaysia. Amongst them, V36 is one of the main accessions in seed and fiber production in Malaysia; therefore it was selected as the control accession in present study to ensure better selection of accessions. Approximately 100 g of seeds with three replications (100 seeds x 3 replications: 300 seeds) for each accession were prepared for seed moisture content (oven-dry method), and 100 seeds with three replications (100 seeds x 3 replications x 2 methods: 600 seeds) were used for germination tests for each accession as recommended by the International Standards for Genebanks (Rao *et al.* 2006). The top of paper method and between paper method (ISTA 2005) were used in the presence of basic requirements for seed germination such as water, oxygen, light, and suitable temperature.

The seeds for field trials were soaked (pre-treatment) in a 400 mL beaker (Pyrex Iwaki TE-32 Asahi Glass; Pt. Iwaki Glass, Sumedang, Indonesia) filled with distilled water for 24 h before being sown at the experimental site to improve germination and seedling growth (Donovan 2001). The evaluation of seedlings was performed according to specific criteria listed by International Seed Testing Association (ISTA) (2005) and Association of Official Seed Analysts (AOSA) (2016). The accession names, origin, seed moisture content, and seed germination results are tabulated in Table 1.

Table 1. Accession Name, Origin, Seed Moisture Content, and Seed Germination of Nine Selected *H. cannabinus* Accessions

No.	Accession	Origin	Seed Moisture Content (%)	50% Normal Seed Germination
1.	V36 (control)	NKTB, Kelantan, Malaysia	12.52 ± 0.02	39.67 ± 1.53
2.	FH952	IBFC, Changsha, China	9.17 ± 0.06	40.00 ± 1.53
3.	T15	IBFC, Changsha, China	10.06 ± 0.06	45.00 ± 2.33
4.	T17	IBFC, Changsha, China	9.71 ± 0.02	41.83 ± 1.20
5.	T19	IBFC, Changsha, China	9.71 ± 0.11	44.83 ± 1.67
6.	HC2	BJRI, Dhaka, Bangladesh	14.73 ± 0.06	21.67 ± 3.06
7.	HC95	BJRI, Dhaka, Bangladesh	13.29 ± 0.01	44.17 ± 0.33
8.	V4202	BJRI, Dhaka, Bangladesh	13.66 ± 0.08	30.83 ± 2.31
9.	V4383	BJRI, Dhaka, Bangladesh	14.05 ± 0.10	27.17 ± 0.33

BJRI = Bangladesh Jute Research Institute; IBFC = Institute of Bast Fiber Crops China; NKTB = National Kenaf and Tobacco Board

Study Site and Experimental Design

The study site was established at University Agricultural Park, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (2° 58’ N latitude, 101° 39’ E longitude), with an area of 26 m × 10 m. It receives up to 2140 mm mean annual precipitation, 26 °C mean annual temperature, and 5 h to 8 h average radiance. The study was conducted from July 2017 to October 2017.

The experiment was arranged using a randomized complete block design. The randomization was assigned using a table of random numbers (Gomez and Gomez 1984) with three replications of planting block. Each block consisted of nine accessions with 10 plants for each accession. The individual plants were arranged with 30 cm planting distance, with 1 m between subblocks and 2 m between blocks. The total number of plants were 270 (10 plants x 9 accessions x 3 replications). Approximately 5 g of Nitrophoska standard formulation fertilizer (15:15:15) (EuroChem, Zug, Switzerland) was supplied manually for each plant on a monthly basis. The insecticide (Kencis with 5.5% Emulsifiable Concentration (EC) of cypermethrin active ingredient; Kenso Corporation, Petaling Jaya, Malaysia) in mixture of 13 L of water and 0.013 L of insecticide (0.01% concentration) was periodically applied twice per month to keep the plants healthy as guided by the Pesticide Control Division of the Department of Agricultural Malaysia.

Soil Sample Analysis

The soil samples were taken from the depth of 0 to 15 cm and 15 to 30 cm using the zig-zag pattern for the whole (Ackerson 2018) experimental site. The samples were air-dried for one week, ground, and sieved through a 2 mm pore size sieve. Soil particle-size analysis was conducted by the relative quantities of sand, silt, and clay using pipette method (Gee and Bauder 1986). The time and depth of the sampling are deduced using Stoke's Law (Stokes 1849). Soil classification were then classified according to scheme developed by USDA (United States Department of Agriculture) in which the soil particles and their sizes ranges are: sand (50 to 2000 µm), silt (2 to 5 µm), and clay (< 2 µm).

Soil texture was determined by using the texture software by Teh and Rashid (2003). Soil pH was measured in soil/ water (1: 2.5) suspension. Total carbon and total nitrogen were measured by CNS analyzer machine (model: LECO TruMac CNS Analyzer). The available phosphorus (P) was determined by Bray-II method as described by Kuo (1996). The exchangeable potassium (K), calcium (Ca), and magnesium (Mg) were determined using an autoanalyzer (QuikChem, Series 8000, Lachat Instruments Inc., USA), and the concentrations of calcium (Ca) and magnesium (Mg) were also determined by atomic absorption spectrophotometer (Perkin-Elmer 5100 PC) (Hazma *et al.* 2015). The initial physical and chemical properties of the soil are presented in Table 2.

Table 2. Initial Soil Physical and Chemical Properties at the Experimental Site

Soil depth (cm)	pH	Soil texture (%)			Total N (%)	Av. P (µg/ g)	Exchangeable (µg/ g)		
		Sand	Clay	Silt			K	Ca	Mg
0-15	4.28 ± 0.07	51.84 ± 1.7	27.45 ± 0.2	20.71 ± 1.5	0.08 ± 0.00	Traces	29.93 ± 5.8	103.18 ± 15.87	16.87 ± 5.50
15-30	4.30 ± 0.08	51.58 ± 0.3	28.86 ±0.5	19.56 ± 0.6	0.05 ± 0.00	Traces	22.76 ± 5.2	83.19 ± 11.69	9.3 ± 2.06

Note: % of N = 10,000 µg/g

Methods

Morphophysiological characteristics

The data on quantitative morphological, physiological, and yield characteristics were assessed according to the Sustainable Projects Development Group of UK and the literature (Pace *et al*. 1998; Ahmad *et al.* 2001) in replicates of 30 specimens for each accession. Both quantitative characteristics (morphological and physiological) were measured monthly using specific measuring equipment, and yield characteristics were recorded after harvest (Table 3).

Laboratory sample preparation

Alkaline treatment involved immersing the fibers in an alkaline solution for a period of time. It was believed that alkaline-treated fibers provide higher tensile modulus than the untreated fibers (Li *et al.* 2007). In the present study, sodium hydroxide (NaOH) and sodium sulfite, anhydrous (Na_2SO_3) were used to remove surface impurities (lignin, wax, and oil covering the external surface of fiber cell wall), while acetic acid (CH_3COOH) was used in retaining a high index of crystallinity (Mohanty *et al.* 2000) and giving the whitest color of fiber (Hurren *et al.* 2002).

Table 3. Description of the Measured Quantitative Characteristics of Nine Selected *H. cannabinus* Accessions

Characteristics	Denotation	Description
Stem Height (cm)	H	Height of each plant was measured from soil surface to the tip of the plant using 5 m x 25 mm measuring tape (ST33463 Stanley, Black & Decker, Inc., Towson, MD, USA)
Stem Diameter (mm)	D	Diameter of stem was taken 5 cm from the base of the plant stalk using an Absolute Digimatic caliper (Mitutoyo, Kawasaki, Japan).
Leaf Area (cm^2)	A_L	Harvested leaf component was taken for leaf areas (cm^2) measurement using leaf area meter (Li-3100C; LiCor Inc., Lincoln, NE, USA).
Stem Dry Weight (g)	SDW	Plant sample was harvested after 120 d plantation and separated to each component listed for oven-drying (U40; Memmert, Schwabach, Germany) process at 70 °C for 48 h, until a constant weight was obtained by using a standard weighing scale (DIGI DS-425; Teraoka Seiko Co., Ltd., Tokyo, Japan).
Fruit Dry Weight (g)	FDW	
Leaf Dry Weight (g)	LDW	
Root Dry Weight (g)	RDW	
Total Aboveground Dry Weight (g)	TAGDW	Total aboveground biomass was calculated by adding values of SDW, FDW, and LDW.
Growth Efficiency (g cm^{-2} d^{-1})	E_G	Referred to as the efficiency of stem growth by ratio of stem dry weight absolute growth rate (AGR) to leaf area (A_L) (Tschieder *et al.* 2012): $\mathrm{E_G} : \frac{\mathrm{AGR}}{\mathrm{A_L}}$
Photosynthesis Rate (μmol m^{-2} s^{-1})	A_{net}	Photosynthesis rate, stomatal conductance, intercellular concentration of carbon dioxide, and transpiration rate were recorded monthly using an open gas exchange system, the LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The measurement was performed on three leaves per plant and three plants for each block replicate and accession [n = 3 leaf x 3 block x 9 accession (n = 81)]. The period of time was randomized in the range 0800 h to 1100 h in each accession for every month (Senin 2016).
Stomatal Conductance (mol m^{-2} s^{-1})	G_S	
Intercellular Concentration of Carbon dioxide (μmol/mol)	C_i	
Transpiration Rate (mmol m^{-2} s^{-1})	E_L	
Water Use Efficiency	WUE	To define the efficiency of plants bringing in carbon dioxide for photosynthesis without losing much water out of its stomata, measured by instantaneous water use efficiency (WUE_{inst}) and intrinsic water use efficiency (WUE_i) derived from parameters of gas exchange measurement (Fischer and Turner 1978): $\mathrm{WUE_{inst}} : \frac{\mathrm{A_{net}}\ (\mu\mathrm{mol\ m^{-2} s^{-1}})}{\mathrm{E_L}\ (\mathrm{mmol\ m^{-2} s^{-1}})}$ $\mathrm{WUE_i} : \frac{\mathrm{A_{net}}\ (\mu\mathrm{mol\ m^{-2} s^{-1}})}{\mathrm{G_s}\ (\mathrm{mol\ m^{-2} s^{-1}})}$
Carboxylation Efficiency	A_{net}/Ci	Referred to as the efficiency of stem growth by ratio of photosynthesis rate (A_{net}) to intercellular concentration of carbon dioxide (Ci) (Silva *et al.* 2013): $\frac{\mathrm{A_{net}}}{\mathrm{Ci}} : \frac{\mathrm{A_{net}}\ (\mu\mathrm{mol\ m^{-2} s^{-1}})}{\mathrm{Ci}\ (\mu\mathrm{mol\ mol^{-1}})}$

For the retting process, three replications of plant samples for each accession were prepared by cutting the lower part of *H. cannabinus* stem approximately 10 cm above the ground. The sample was peeled manually to separate the bast fiber and core fiber. The bast fiber was cut into 15 cm of length, in weight of 30 g per sample (Amel *et al.* 2013).

The aqueous solution for the retting process was prepared by mixing 7% NaOH and 0.5% Na_2SO_3 solution. The mixed solution was then stirred using a magnetic stirrer (IKA Magnetic Stirrers RH 2 Basic; IKA Works (Asia) Sdn. Bhd., Selangor, Malaysia). The bast fiber was then immersed into the aqueous solution using a narrow mouth conical flask (Pyrex No. 4980 250 mL Erlenmeyer Flask; Corning Inc., New York, USA). It was then soaked in a water bath (Nickel Electro NE4-22T; Thermo Fisher Scientific Inc., Goteborg, Sweden) for 60 min at 100 °C. After the retting process, the sample was neutralized by removing the sample from the aqueous solution, placing filter paper in a glass conical funnel, and rinsing with 2% CH_3COOH (Ramaswamy and Boyd 1994). The unattached lignin, wax, and color were then removed by two dunks in hot plain water at 70 °C. The fiber was then submerged under plain water and dunked 10 times before it was air-dried for two weeks (Hurren *et al.* 2002). The sample was then used for fiber diameter measurement and tensile strength testing.

Fiber diameter determination

SEM imaging was conducted at the Laboratory of Biopolymer and Derivatives, Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia (Selangor, Malaysia). A small part of sample retted above was taken for fiber diameter and surface morphology study. Three replications for each accession were cut into widths and lengths of 2 mm. The bundle of fiber (to increase data taken of the image instead of using single fiber) for each sample were placed into aluminium specimen mounts with slotted head 12.7 mm with tapered end pin 3.1 mm (Electron Microscopy Sciences, Hatfield, United States). The sample was vacuumed and coated with a thin layer of conductive material (gold and platinum) by using SPT-20 Ion Sputter Coater (COXEM, Daejeon, Korea) to prevent electron build up degrading the image quality. The coated sample were then inserted into a motorised chamber of 3-axis XYT sample position stage of scanning electron microscopy (SEM) (EM-30AX Plus; COXEM, Daejeon, Korea). The navigated sample was then extracted by using COXEM Nanostation operating software. The fiber diameter was then calculated as an average of three measurements of the single fiber.

Fiber tensile testing

Five grams of *H. cannabinus* bast fiber bundle in three replicates for each accession were used for tensile test determination. The test was conducted according to ASTM D885 (1995). The tensile test was done at the Strength Material Laboratory, Faculty of Engineering, Universiti Putra Malaysia (Selangor, Malaysia). A dual column tabletop universal testing machine (INSTRON 3365; Instron, Norwood, MA, USA) was used, with a cell load capacity of 5 kN, at a crosshead speed of 1 mm/min with a gauge length of 60 mm. The standard specifics of sample preparation was followed Amel *et al.* (2013). 80 gsm A4 paper size (IK Yellow; Goldtech Access Sdn Bhd, Selangor, Malaysia) was cut in similar width (7 cm) of the screw grips and in length 12 cm. A rectangle-shape-hole was made at the center of the paper in width and length of 3 cm and 6 cm (effective gauge length of 6 cm). Both end of the sample was then glued to the paper (3 mm width and 30 mm length at both end of the sample) to avoid the possibility of a sample becoming fractured or slipping in the gripped area.

The lower and upper clamp with smooth face of interchangeable jaws face (Model 2710-004 side-acting screw grips; Instron, Norwood, MA, USA) was adjusted to accommodate the length of the sample. The glued part of the sample was vertically mounted from the lower clamp (the fixed grip) to the upper clamp (the grip in charge of applying tension). The clamp was manually tightened at both knobs at sides of the grips. The tensile strength determination was focused on the gauge length, in which one grip keeps the sample in place, while the other grip pulled at a constant speed until the sample fractured. Once the sample had broken, the tensile testing has officially ended. The tensile properties were recorded as a function of the increase in gauge length (sample elongation during applying tension until fractured). A 5 mm increase in gauge length approximately yields a 20% decrease in fiber strength (Ramesh 2016). Tensile properties of the specimen were then analysed by using Instron Bluehill 3 software (Instron, Norwood, MA, USA).

Statistical analysis

All measurements were analyzed using a factorial analysis of variance (ANOVA) and mean comparison *via* Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ (significant difference) and $p \leq 0.01$ (highly significant difference). The relationship between the morphophysiological and fiber characteristics were analyzed using bivariate (Pearson) correlation analysis. Interpretation of the correlation coefficient values was based on Ratner (2009). The growth performance of morphological characteristics namely stem height and stem diameter were also evaluated using Simple Scatterplot. The statistical analyses were conducted using IBM SPSS statistics software version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Growth Performance

Stem height and stem diameter are considered as the general guiding criteria for efficient production of fibers in a particular species (Maiti and Chakravarty 1977). Figures 1 and 2 demonstrate growth patterns of the nine *H. cannabinus* accessions for the four-month cultivation regarding stem height and stem diameter increment, respectively.

Regardless of accession, the stem height increments at the first month after planting (MAP) were in the range from 29.3 to 44.1 cm, and the increment at the fourth MAP were from 43.0 to 55.7 cm. The V36 accession (control), T19 (IBFC China accession), and all four BJRI Bangladesh accessions exhibited strong growth for the first three MAP and slowed down at the fourth MAP, which resulted in higher growth (Table 4) than the other (FH952, T15, and T17) IBFC China accessions, which were slowed down after the second MAP (Fig. 1a). Salih *et al.* (2014), however, found that plant growth of FH952 (stem height and stem diameter) was greater than the V4383 accession. Meanwhile Sultana *et al.* (2016) recorded shoot regeneration of HC2 taking minimum days (4.71 days) compared to HC95 accession (7. 41 days).

All nine accessions were found to produce the first bud at 10^{th} to 15^{th} node (at stem height 50 to 60 cm) with a minimum of stem height of 70 cm. The first flowering was formed on the 10^{th} to 15^{th} node (at stem height 80-90 cm) with minimum stem height was 100 cm. The vegetative growth of all accessions were continued with time.

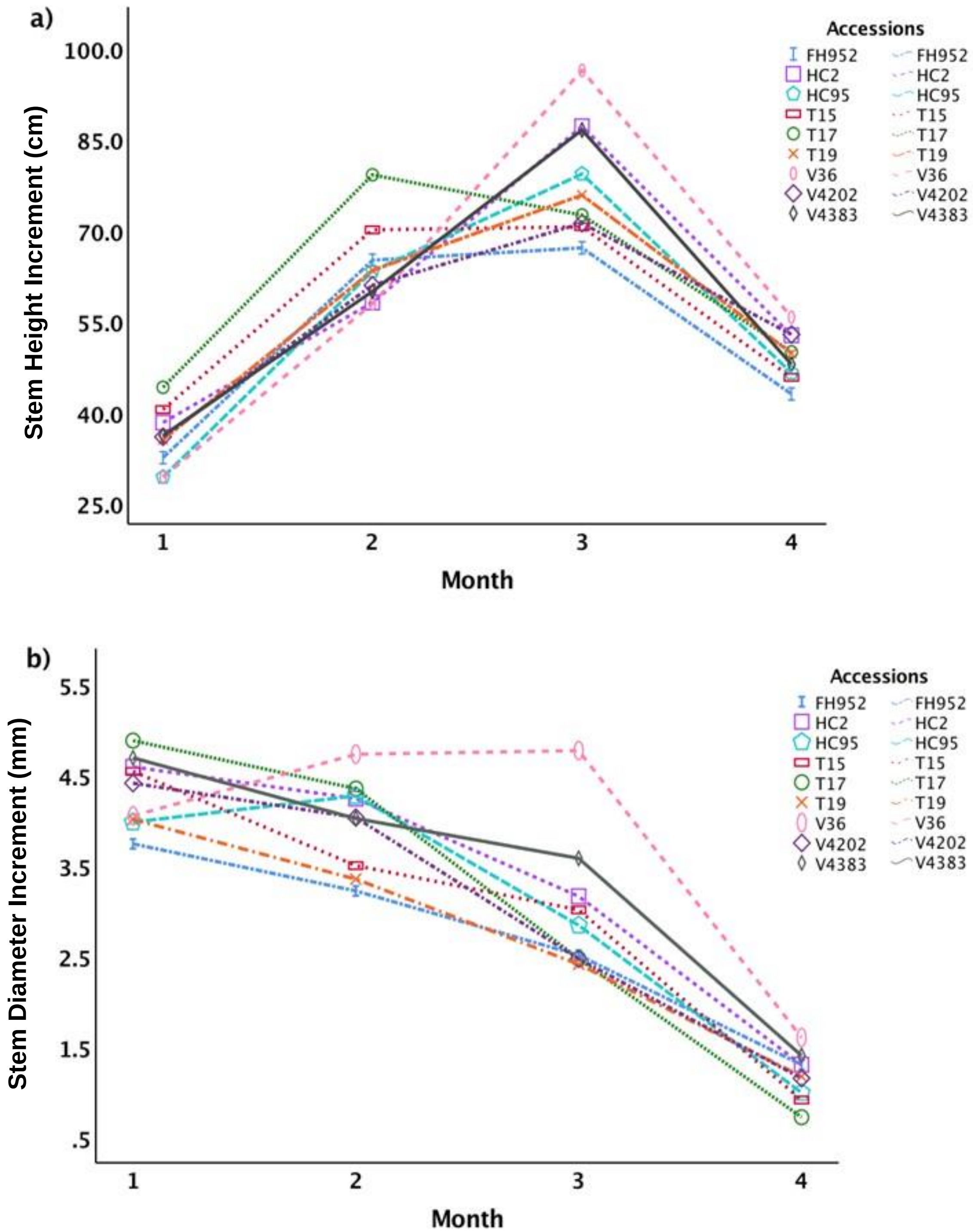


Fig. 1. Growth performance of nine *H. cannabinus* accessions; a) stem height increment (cm), b) stem diameter increment (mm) of four month of planting

Stem diameter increment at the first MAP were found in the range from 3.74 to 4.88 mm, while at fourth MAP were found in range from 0.72 mm to 1.60 mm. Stem diameter increment of control accession were observed to decreased considerably only after three MAP compared to other accessions (Fig. 1b). HC95 accession resulted in higher stem diameter increment on the second MAP compared to the first MAP. T15 and V4383 accessions show the same pattern in which stem diameter increment were decreased slightly after second MAP. Meanwhile stem diameter increment of T17, T19, FH952, HC95, HC2, and V4202 accessions were decreased considerably after second MAP.

Most of the control accession plants do not produce buds even after two MAP, compared to other accession in which already produce many buds after two MAP. Ab Shukor *et al*. (2009) found 50% flowering of V36 accession (control) was 83 to 136 days after planting. Hossain *et al.* (2012) found kenaf biomass accumulation such as leaf, root, stalk, and wood dry weight was started at 6th week of growth after planting. Among the nine accessions, the control, V4383, and HC2 accession had the greatest stem diameters, stem dry weights, and total aboveground dry weights, so therefore they portrayed a maximum fiber yield. The results of V36 and HC2 accessions agreed with the findings of Hossain *et al.* (2014; 2016) and Nasreen *et al.* (2014) that the accession produced the highest fiber yield.

Morphological and yield characteristics

The results in Table 4 demonstrate all nine accessions allocated more dry matter to shoots than roots. Similar climatic-condition in present study but with different soil properties produced similar results on the control accession plant height (present study (239 cm), Ab Shukor *et al*. (2009) (240 cm), Omalsaad *et al.* (2012) (212 cm), and Khalatbari *et al*. (2015) (231 cm)).

Plant height was found to be significantly different ($p \leq 0.05$) among nine accessions. The T17 accession recorded the highest stem heights (245.8 cm) followed by control accession (239 cm), and HC2 accession (236 cm), while HC95 accession had the lowest stem height (216 cm). Hossain *et al.* (2014) however recorded HC2 as the higher plant height compared to V36 (control) and HC95 accessions. Lower range of stem height in present study (208 to 246 cm) could be due to our planting distance (30 cm) between individual plants.

Results (Table 4) also indicate that there were highly significantly differences ($p \leq 0.01$) in stem diameter, root dry weight, leaf dry weight, leaf area, and growth efficiency among nine accessions. The control accession showed the largest stem diameter, stem dry weight, and total aboveground dry weight (15.2 mm, 75.7 g, and 122.3 g, respectively), followed by V4383 (13.8 mm, 63.5 g, and 119.0 g, respectively) and HC2 (13.3 mm, 62.6 g, and 117 g, respectively). Meanwhile the lowest stem diameter and stem dry weight were observed for FH952. The stem diameter of the control accession in the present study (15.2 mm) was much smaller than Omalsaad *et al*. (2012) (8.00 cm), but almost similar to Ahmad (2011) (21.6 mm), Hossain *et al.* (2014) (12.0 mm), and Khalatbari *et al*. (2015) (14.1 mm).

Hossain *et al.* (2011) found that HC2 produced on average 2 g per plant higher of stem dry weight, root dry weight, and leaf dry weight compared to V36 (control) accession that was grown on 96.4% sand content of soil. HC95 meanwhile produced the lowest biomass (stem, leaf, and root dry weight) in comparison to HC2 and V36 (Hossain *et al.* 2011; 2016). Stem dry weight FH952 in this finding was in contrast with Qi *et al.* (2002), who reported that FH952 yield was 15.7% more than the standard cultivar in China. Regardless of accession, fruit dry weight was found to be in the range 15.8 g to 31.9 g. Result (Table 4) indicates that fruit dry weight was not significantly different among the nine accessions.

Table 4. ANOVA and Duncan's Multiple Range Test of Quantitative Morphological and Yield Characteristics of Nine *H. cannabinus* Accessions

Accession	*H* (cm)	*D* (mm)	SDW (g)	FDW (g)	RDW (g)	LDW (g)	TABG (g)	A_L (cm^2 $plant^{-1}$)	E_G
V36 (control)	239.33 ± 5.66^{a,b}	15.15 ± 0.35^{a}	75.73 ± 18.00^{a}	29.85 ± 2.50^{a}	17.84 ± 1.77^{b}	16.75 ± 2.54^{d}	122.34 ± 19.08^{a}	342.10 ± 49.44^{c,d}	0.0117 ± 0.001^{b,c,d}
FH952	207.80 ± 9.22^{c}	10.77 ± 0.49^{e}	45.12 ± 5.36^{b}	22.21 ± 2.13^{a}	13.02 ± 0.95^{b}	21.50 ± 3.29^{c,d}	88.84 ± 7.04^{a}	266.11 ± 26.98^{c,d}	0.0090 ± 0.001^{c,d}
T15	226.93 ± 9.04^{a,b,c}	11.96 ± 0.43^{c,d,e}	49.46 ± 4.57^{b}	15.78 ± 1.39^{a}	14.55 ± 1.01^{b}	22.09 ± 1.68^{b,c,d}	87.33 ± 6.55^{a}	455.81 ± 85.09^{c}	0.0138 ± 0.003^{a,b,c}
T17	245.80 ± 7.00^{a}	12.42 ± 0.31^{b,c,d}	54.29 ± 3.72^{a,b}	23.83 ± 1.53^{a}	15.94 ± 1.16^{b}	21.35 ± 1.57^{c,d}	99.50 ± 5.19^{a}	704.16 ± 100.24^{b}	0.0061 ± 0.000^{d}
T19	224.47 ± 7.64^{a,b,c}	10.94 ± 0.45^{d,e}	46.16 ± 4.24b^{b}	17.60 ± 1.11^{a}	14.48 ± 1.47^{b}	22.33 ± 1.58^{b,c,d}	86.51 ± 5.31^{a}	155.30 ± 37.85^{d}	0.0187 ± 0.001^{a}
HC2	236.33 ± 7.04^{a,b}	13.29 ± 4.35^{b,c}	62.56 ± 4.38^{a,b}	29.39 ± 2.61^{a}	18.26 ± 1.99^{b}	25.12 ± 1.57^{a,b,c}	117.06 ± 7.00^{a}	795.89 ± 111.60^{a,b}	0.0067 ± 0.001^{d}
HC95	215.46 ± 9.10^{b,c}	11.93 ± 0.62^{c,d,e}	48.83 ± 7.71^{b}	24.67 ± 2.23^{a}	18.80 ± 2.78^{b}	18.50 ± 2.08^{c,d}	88.92 ± 9.71^{a}	217.20 ± 29.33^{c,d}	0.0145 ± 0.002^{a,b,c}
V4202	222.08 ± 11.26^{a,b,c}	12.28 ± 0.45^{b,c,d,e}	52.51 ± 6.07^{a,b}	31.89 ± 15.35^{a}	16.32 ± 1.68^{b}	29.85 ± 3.42^{a}	114.26 ± 20.56^{a}	261.13 ± 57.62^{c,d}	0.0162 ± 0.002^{a,b}
V4383	229.45 ± 10.94^{a,b,c}	13.79 ± 0.57^{a,b}	63.48 ± 7.59^{a,b}	26.52 ± 6.28^{a}	32.48 ± 11.61^{a}	29.00 ± 2.50^{a,b}	118.95 ± 11.92^{a}	1032.41 ± 206.23^{a}	0.0093 ± 0.002 ^{c,d}
ANOVA (Between Accessions)	*	**	ns	ns	**	**	ns	**	**

Note: Mean values with the same letter in the same row at each factor are not significantly different at $p \leq 0.05$; H = stem height; D = stem diameter; SDW = stem dry weight; FDW = fruit dry weight; RDW = root dry weight; LDW = leaf dry weight; TABG = total aboveground dry weight; A_L = leaf area; E_G = Growth efficiency; ** = highly significantly different at $p \leq 0.01$; * = significantly different at $p \leq 0.05$; ns = not significant

The highest root dry weight was found from V4383 (32.5 g), followed by HC95 (18.8 g) and HC2 (18.3 g), while the lightest was found from FH952 (13.0 g). Hossain *et al.* (2011) reported HC2 (11.85 g) had heavier root dry weight compared to V36 (control) (11.5 g) and HC95 (10.8 g) accessions. Ab Shukor *et al.* (2009) recorded that V36 (control) accession was among the heaviest root dry weight (10.7 g), in which V36 (control) in present study was heavier than reported by Ab Shukor *et al.* (2009) and Hossain *et al.* (2011). A highly significant difference ($p \leq 0.01$) was found for leaves dry weight among accession. The V4202 produced the heaviest leaves dry weight (29.8 g), followed by V4383 (29.0 g) and HC2 (25.1 g), and the lightest leaves dry weight was the control accession (16.8 g). Hossain *et al.* (2011, 2016) reported that HC2 had higher leaves dry weight compared to V36 (control) and HC95 accessions.

The results in Table 4 also showed that leaf area and growth efficiency were highly significantly different ($p \leq 0.01$) among nine accessions. The biggest average leaf area was found in V4383 (1032 cm^2 $plant^{-1}$), followed by HC2 (796 cm^2 $plant^{-1}$) and T17 (704 cm^2 $plant^{-1}$) accessions, and the lowest leaf areas was T19 accession (155 cm^2 $plant^{-1}$). Hossain *et al.* (2016) recorded leaf area of HC2 and V36 (control) was 900 cm^2 $plant^{-1}$. However, the highest growth efficiency was found in T19 (0.0187 g cm^{-2} d^{-1}) while the lowest was T17 (0.0023 g cm^{-2} d^{-1}).

Physiological characteristics

Photosynthesis is the process in which the energy from light is used to synthesize carbon compounds in foliage leaf (Pallardy 2008; Weraduwage *et al*. 2015). The physiological characteristics, such as photosynthesis rate (A_{net}), stomatal conductance (G_s), intercellular concentration of carbon dioxide (Ci), transpiration rate (E_L), instantaneous water use efficiency (WUE_{inst}), intrinsic water use efficiency (WUE_i), and carboxylation efficiency (A_{net}/Ci), were measured in this study.

The results (Table 5) indicate that all physiological characteristics were significantly different ($p \leq 0.05$) among *H. cannabinus* accessions, as well as the control accession. The highest A_{net} was found from HC2 (35.1 μmol m^{-2} s^{-1}) followed by T17 (35.0 μmol m^{-2} s^{-1}), and control (33.10 μmol m^{-2} s^{-1}) accessions, while the lowest rate was found from V4202 accession (27.4 μmol m^{-2} s^{-1}). A_{net} of the control accession in the present study was higher than reported by Khalatbari (2016) at 21.6 μmol m^{-2} s^{-1} and Hossain *et al.* (2016) at 9.9 μmol m^{-2} s^{-1}. Hossain *et al.* (2016) also reported that the A_{net} of HC2 was 10.11 μmol m^{-2} s^{-1}. High A_{net} has been suggested to contribute to high amount of dry matter production (Table 4) (Salih *et al.* 2014; Hossain *et al.* 2016).

The highest G_s was found from HC2 (1.08 μmol m^{-2} s^{-1}), followed by control (1.03 μmol m^{-2} s^{-1}), HC95 (0.85 μmol m^{-2} s^{-1}), and the lowest recorded by T17 accession (0.78 μmol m^{-2} s^{-1}). Khalatbari *et al.* (2016) reported a G_s of V36 (control) of 1.00 μmol m^{-2} s^{-1}, which was similar to the present findings. The T17 accession meanwhile might possess better control of stomatal function in water deficit conditions. The highest Ci was found from the FH952 (339.64 μmol m^{-2} s^{-1}) followed by V4202 (337.03 μmol m^{-2} s^{-1}), V36 (334.77 μmol m^{-2} s^{-1}), and the lowest recorded by the V4383 (294.53 μmol m^{-2} s^{-1}) accession.

The highest E_L was recorded by HC2 (6.26 mmol m^{-2} s^{-1}), followed by V4202 (5.87 mmol m^{-2} s^{-1}) and control (5.70 mmol m^{-2} s^{-1}), while the lowest was from V4383 (4.54 mmol m^{-2} s^{-1}). Khalatbari *et al*. (2016) recorded that the E_L of V36 (control) was 1.88 mmol m^{-2} s^{-1}, and 1.71 mmol m^{-2} s^{-1} by Tahery (2011), which was much lower compared to the present study. The highest WUE_{inst} was found from T17 (7.61 μmol $mmol^{-1}$), followed by V4383 (7.50 μmol $mmol^{-1}$), and T15 (6.87 μmol $mmol^{-1}$), while the lowest was found from V4202 accession (4.88 μmol $mmol^{-1}$). The V4383 accession had the highest WUE_i (61.9 μmol mol^{-1}), followed by T17 (50.1 μmol mol^{-1}), T15 (43.0 μmol mol^{-1}), and the lowest was HC2 accession (32.8 μmol mol^{-1}).

Table 5. ANOVA and DMRT of Physiological Characteristics of Nine *H. cannabinus* Accessions

Accession	A_{net} (μmol m^{-2} s^{-1})	G_s (mol m^{-2} s^{-1})	Ci (μmol mol^{-1})	E_L (mmol m^{-2} s^{-1})	WUE_{inst} (μmol mmol^{-1})	WUE_i (μmol mol^{-1})	A_{net}/Ci (μmol m^{-2} s^{-1}/μmol mol^{-1}
V36 (control)	33.10 ± 0.77^{a,b}	1.03 ± 0.03^{a}	334.77 ± 1.63^{a,b}	5.70 ± 0.14^{a,b}	5.85 ± 0.13^{b}	32.76 ± 0.85^{c}	0.10 ± 0.00^{b,c}
FH952	27.80 ± 0.86^{c,d}	0.82 ± 0.04^{b}	339.64 ± 2.32^{a}	5.61 ± 0.13^{a,b}	4.95 ± 0.09^{b}	34.77 ± 1.15^{c}	0.08 ± 0.00^{d}
T15	32.46 ± 1.50^{a,b}	0.78 ± 0.37^{b}	319.91 ± 6.93^{b}	4.80 ± 0.12^{c}	6.87 ± 0.42^{a}	42.95 ± 3.24^{b,c}	0.10 ± 0.00^{a,b,c}
T17	35.00 ± 1.23^{a}	0.77 ± 0.04^{b}	303.98 ± 9.98^{b}	5.02 ± 0.26^{b,c}	7.61 ± 0.72^{a}	50.13 ± 5.29^{a,b}	0.12 ± 0.00^{a}
T19	29.40 ± 0.90^{b,c,d}	0.80 ± 0.03^{b}	332.81 ± 1.51^{a,b}	5.66 ± 0.15^{a,b}	5.23 ± 0.14^{b}	37.18 ± 0.75^{c}	0.09 ± 0.00^{c,d}
HC2	35.11 ± 0.57^{a}	1.08 ± 0.02^{a}	330.91 ± 1.22^{a,b}	6.26 ± 0.12^{a}	5.65 ± 0.11^{b}	32.78 ± 0.40^{c}	0.12 ± 0.00^{a,b}
HC95	31.91 ± 1.51^{a,b,c}	0.85 ± 0.51^{b}	326.74 ± 3.94^{a,b}	5.57 ± 0.28^{a,b}	5.84 ± 0.31^{b}	38.63 ± 1.88^{c}	0.10 ± 0.00^{b,c,d}
V4202	27.43 ± 1.49^{d}	0.81 ± 0.05^{b}	337.03 ± 2.81^{a}	5.87 ± 0.31^{a}	4.88 ± 0.28^{b}	35.89 ± 2.13^{c}	0.08 ± 0.00^{d}
V4383	32.73 ± 3.26^{a,b}	0.82 ± 0.09^{b}	294.53 ± 12.63^{a}	4.54 ± 0.43^{c}	7.50 ± 0.42^{a}	61.94 ± 10.04^{a}	0.11 ± 0.00^{a,b}
ANOVA (between accessions)	*	*	*	*	*	*	*

Note: Mean values with the same letter in the same row at each factor are not significantly different at $p \leq 0.05$; A_{net} = photosynthesis rate; G_s = stomatal conductance; Ci = intercellular concentration of carbon dioxide; E_L = transpiration rate; WUE_{inst} = instantaneous water use efficiency; WUE_i = intrinsic water use efficiency; A_{net}/Ci = carboxylation efficiency

Tensile strength and fiber diameter

Fiber diameter was significantly different among *H. cannabinus* accessions, including the control (Table 6). The largest fiber diameter (32.6 µm) on average was from the V4202 accession, followed by the T15 (30.75 µm) and T17 (25.05 µm) accessions. Khalatbari *et al.* (2016) reported fiber diameter of V36 (control) accession was 23.0 µm. Meanwhile H'ng *et al*. (2009) recorded 24.1 µm, which is similar to this finding. The results also indicated that the fiber tensile modulus of nine *H. cannabinus* accessions ranged from 10.8 MPa to 77.9 MPa. The three highest tensile modulus values on average were observed from the V4383 (77.9 MPa), FH952 (52.2 MPa), and HC2 (52.0 MPa) accessions, whereas the control accession had an average value of 35.2 MPa.

The three lowest tensile modulus values were observed from the HC95 (16.5 MPa), V4202 (14.9 MPa), and T15 (10.8 MPa) accessions. Both the fiber diameter and tensile modulus were significantly different ($p \leq 0.05$) among the accessions. The V4383 accession had a smallest fiber diameter (10.9 µm) with the highest tensile modulus (77.9 MPa). Meanwhile, the V4202 accession had the biggest fiber diameter (32.6 µm) with low tensile modulus (14.9 MPa).

Table 6. Fiber Tensile Strength and Diameter of Nine *H. cannabinus* Accessions

Accession	Fiber Diameter (µm)	Tensile Modulus (MPa)
V36 (control)	$24.03^{b,c} \pm 0.49$	$35.20^{b,c} \pm 5.86$
FH952	$17.50^{d,e} \pm 1.71$	$52.20^{a,b} \pm 6.23$
T15	$32.53^{a} \pm 1.81$	$10.84^{c} \pm 0.75$
T17	$26.07^{b} \pm 1.04$	$19.28^{c} \pm 1.44$
T19	$20.67^{c,d} \pm 0.91$	$31.19^{b,c} \pm 2.86$
HC2	$13.27^{d} \pm 0.52$	$52.03^{a,b} \pm 9.90$
HC95	$16.27^{d,e} \pm 2.29$	$16.53^{c} \pm 2.89$
V4202	$35.43^{a} \pm 2.89$	$14.91^{c} \pm 3.92$
V4383	$10.90^{f} \pm 0.15$	$77.87^{a} \pm 23.58$
ANOVA (Between accessions)	*	*
Note: Mean values with the same letter in the same row at each factor are not significantly different at p ≤ 0.05		

Correlation Analysis

Morphological characteristics

A highly significant ($p \leq 0.01$) strong positive relationship ($r = 0.790$) was found between stem height and the stem diameter, a moderate positive relationship with stem dry weight ($r = 0.556$), total aboveground dry weight ($r = 0.590$), fruit dry weight ($r = 0.332$), root dry weight ($r = 0.362$), and a weak relationship with leaf dry weight ($r = 0.183$) (Table 7). A significant ($p \leq 0.05$) but weak relationship ($r = 0.152$) was observed between plant height and leaf area. This study found a significant relationship between stem diameters and all morphological and yield characteristics except for leaf dry weight and growth efficiency. Meanwhile there was no significant relationship found between stem height and growth efficiency. Among all morphological characteristics, the strongest ($p \leq 0.01$; $r = 0.848$) relationship was found between stem dry weight and total aboveground dry weight.

Stem dry weight was found significantly related with fruit dry weight, root dry weight, total aboveground dry weight, leaf area, but negatively related with growth efficiency. Fruit dry weight meanwhile was significantly associated only with leaf dry weight ($p \leq 0.01$; $r = 0.217$) and total aboveground dry weight ($p \leq 0.01$; $r = 0.682$). Root dry weight and leaf dry weight increases when total aboveground biomass increased ($p \leq 0.01$; $r = 0.205$, 0.373 respectively). Significant positive but weak relationships were found between leaf area with stem height ($p \leq 0.05$; $r = 0.152$), stem diameter ($p \leq 0.01$; $r = 0.273$), stem dry weight ($p \leq 0.01$; $r = 0.218$), and total aboveground biomass ($p \leq 0.01$; $r = 0.205$) depicted increase in leaf area resulted to slightly increases of all above characteristics. Moreover, increasing of leaf area affect to decrease in *H. cannabinus* growth efficiency ($p \leq 0.01$; r= -0.586). The growth efficiency meanwhile decreased ($p \leq 0.05$; $r = -0.158$) with increasing of stem diameter but increased ($r = 0.160$) with increasing of stem dry weight.

Table 7. Combined Analysis for Correlation Coefficient of Quantitative Morphological Characteristics of Nine *H. cannabinus* Accessions

	D	SDW	FDW	RDW	LDW	TABG	A_L	E_G
H	0.790**	0.556**	0.332**	0.362**	0.183**	0.590**	0.152*	-0.038
D		0.590*	0.346**	0.386**	0.112	0.606**	0.273**	-0.158*
SDW			0.245**	0.200**	0.089	0.848**	0.218**	-0.160*
FDW				0.109	0.217**	0.682**	0.109	-0.066
RDW					0.057	0.205**	0.100	-0.082
LDW						0.373**	-0.06	0.122
TABG							0.205**	-0.119
A_L								-0.586**

Note: * = Significant at 0.05 probability level; ** = Highly significant at 0.01 probability level; H = stem height; D = stem diameter; SDW = stem dry weight; FDW = fruit dry weight; RDW = root dry weight; LDW = leaf dry weight; TABG = total aboveground dry weight; A_L = leaf area; E_G = growth efficiency

Physiological characteristics

Increasing of A_{net} values resulted from the increase of G_s ($p \leq 0.01$; $r = 0.619$), thus increasing E_L ($p \leq 0.01$; $r = 0.337$), WUE_{inst} ($p \leq 0.01$; $r = 0.518$), and carboxylation efficiency ($p \leq 0.01$; $r = 0.937$). The Ci ($p \leq 0.05$; $r = -0.144$) and WUE_i ($p \leq 0.01$; r = -2.75) however decreased with increasing of A_{net}. The G_s was found to have a highly significant ($p \leq 0.01$) strong positive relationship ($r = 0.728$) with E_L, and moderate relationship with Ci and carboxylation efficiency.

Table 8. Combined Analysis for Correlation Coefficient of Physiological Characteristics of Nine *H. cannabinus* Accessions

	G_s	Ci	E_L	WUE_{inst}	WUE_i	A_{net}/Ci
A_{net}	0.619**	-0.144*	0.337**	0.518**	-0.275**	0.937**
G_s		0.479**	0.728**	-0.193**	-0.683**	0.346**
Ci			0.628**	-0.815**	-0.905**	-0.428**
E_L				-0.575**	-0.741**	0.094
WUE_{inst}					0.567**	0.739**
WUE_i						0.11

Note: * = Significant at 0.05 probability level; ** = Highly significant at 0.01 probability level; A_{net} = photosynthesis rate; G_s = stomatal conductance; Ci = intercellular concentration of carbon dioxide; E_L = transpiration rate; WUE_{inst} = instantaneous water use efficiency; WUE_i = intrinsic water use efficiency; A_{net}/Ci = carboxylation efficiency

The increasing of G_s depicted decreases in WUE_{inst} ($p \leq 0.01$; r = -0.193) and WUE_i ($p \leq 0.01$; r = -0.683) (Table 8). An increase of Ci meanwhile depicted an increase in E_L of the plant ($p \leq 0.01$; r = 0.628) but a decrease in WUE and carboxylation efficiency. The WUE_{inst} increase with increasing of WUE_i ($p \leq 0.01$; r = 0.567) and carboxylation efficiency ($p \leq 0.01$; r = 0.739) which demonstrated that the increasing of G_s resulted in increases of E_L and Ci, but a decrease in WUE.

Tensile strength and fiber diameter

Large fiber diameter resulted in a low tensile modulus of fiber (Fig. 2). Inacio *et al.* (2010) reported that the smaller the diameter, the stronger is the fiber for several natural fibers such as cotton (*Gossypium hirsutum*), curaua (*Ananas erectifolius*), jute (*Corchorus capsularis*), and sisal (*Agave sisalana*). Weibull analysis of sisal fiber tensile strength conducted by Inacio *et al.* (2010) also shows an inverse dependence of the tensile strength with the diameter.

In the present study, Fig. 2 showed an inverse correlation between fiber strength and fiber diameter. A fractographic analysis by SEM of the *H. cannabinus* bast fiber surface in Fig. 3 depicted the thicker fiber with a diameter of *d* equal to 26.1 µm and above (Table 6). The figure reveals a heterogeneous fracture and covered by a number of impurities (Fig. 3c, 3d, and 3h) suspected to be hemicellulose, lignin, pectin, and waxy substances associated with relatively more fibrils (Tahir *et al.* 2015). In other words, the fiber tensile modulus less than 20 MPa had voids, impurities, and showed fiber damage on the surface (Fig. 3c, 3d, 3g, and 3h).

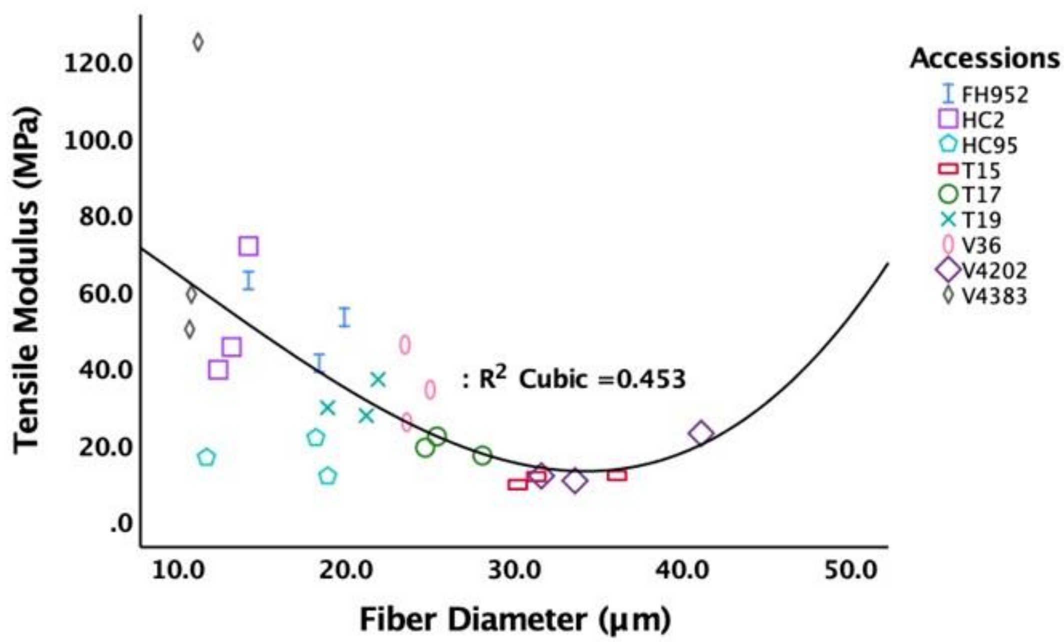
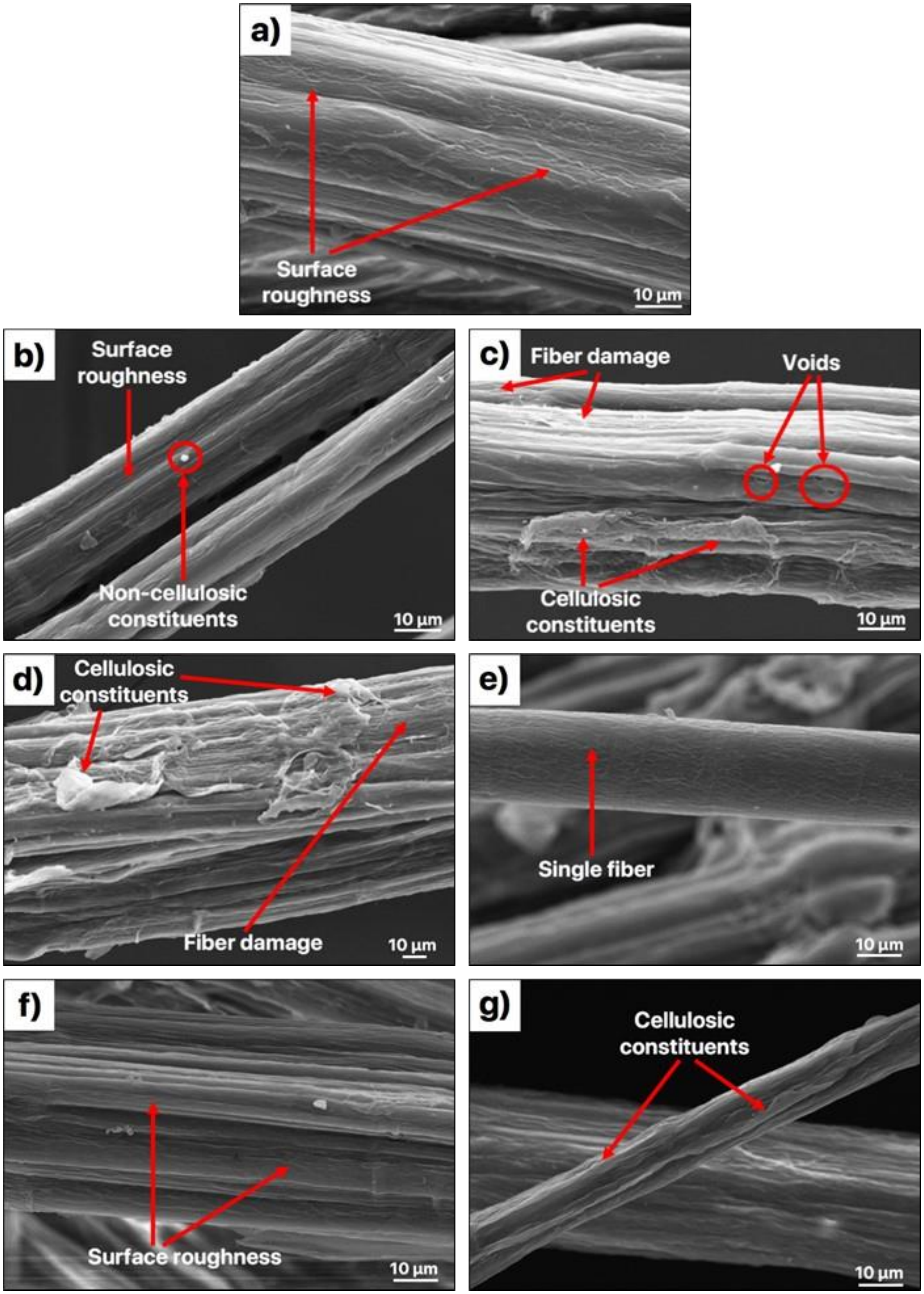


Fig. 2. Correlation of fiber diameter and tensile modulus of *H. cannabinus* accessions


a)
Surface roughness
10 µm
b)
Surface roughness
Non-cellulosic constituents
10 µm
c)
Fiber damage
Voids
Cellulosic constituents
10 µm
d)
Cellulosic constituents
Fiber damage
10 µm
e)
Single fiber
10 µm
f)
Surface roughness
10 µm
g)
Cellulosic constituents
10 µm

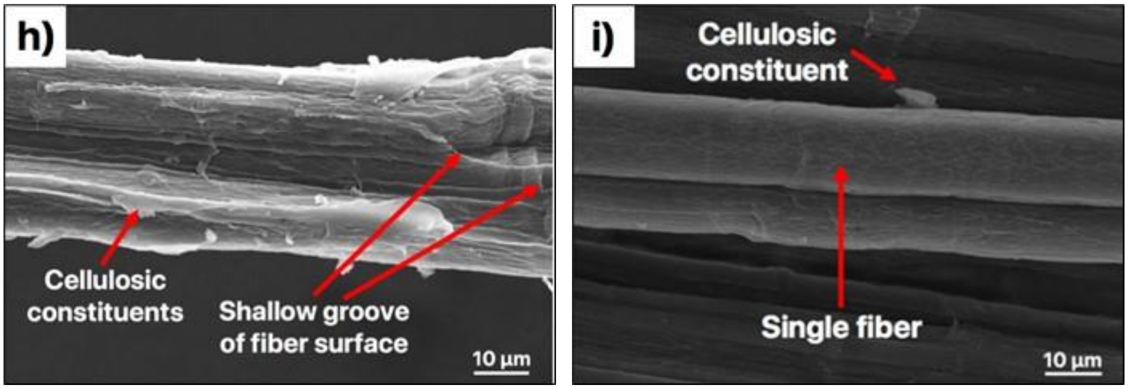


Fig. 3. SEM imagery of nine *H. cannabinus* accessions; a) V36 (control), b) FH952, c) T15, d) T17, e) T19, f) HC2, g) HC95, h) V4202, and i) V4383

Figure (3g) however demonstrated low tensile modulus with low fiber diameter. This could be due to larger internal area of lumen, thinner cell-wall, and low content of cellulose (Fidelis *et al.* 2013). Previous studies (Mohamed *et al.* 2019a,b) showed that fiber lumen diameter and holocellulose of same bamboo species were significantly different ($p \leq 0.01$) from the different study location, but not significantly different on fiber cell wall thickness and alpha-cellulose. In the present study, the fractographic analysis indicated that a thicker fiber could break at a stress lower than that required for a thinner fiber. Wirawan *et al.* (2011) explained that the weak tensile strength could be related to the fiber's ability to transfer loads to one another, where the effect of crack initiation was dominant with low fiber contents compared to the effect of crack inhibition.

DISCUSSION

Morphophysiological Characteristics and Tensile Strength

Quantitative morphological and yield characteristics are the most important traits to consider for fiber yield in breeding line selection (Li and Huang 2013). Petrini *et al.* (1994) and Alexopoulou *et al*. (2000) stated that different cultivars give different responses based on the daylength but stem growth generally declines rapidly following the flower initiation; this likely caused a reduction in vegetative growth of *H. cannabinus.* In other words, *H. cannabinus* grew rapidly in the beginning but gradually decreased its rate of growth. Early decreases of stem height increment of China accessions were indicative of an early flowering period. This demonstrated that flowering initiation of China accessions were earlier than control and Bangladesh accessions (Fig. 1a), thus decreased biomass yield of the plant (Table 4). Such postulation agreed with Alexopoulou *et al.* (2000) that the late-maturing accessions grew taller, exhibited a higher growth rate and developed larger stem diameter as compared to the early maturing ones.

Similar climatic-condition in the present study but with different soil properties, as reported by Ab Shukor *et al.* (2009), Omalsaad *et al.* (2012), Khalatbari *et al*. (2015), produced similar results on the control accession's plant height. Ahmad (2011) found significantly higher of V36 (control) plant height (310 cm) under similar-climatic condition but different soil properties with 75 cm row spacing. Regardless of accession, lower range of stem height in present study (208 to 246 cm) could be due to our planting distance (30 cm) between individual plants. Higher range of stem height (244 cm to 426 cm) with unbranched habit, however, were found by LeMaheiu *et al.* (1991) under dense strands condition.

Furthermore, the highest growth efficiency resulted from the accession with the lowest leaf area and low plant growth (T19). As shown in Table 4, it had thicker leaves compared to other accessions. Within leaves, carbon accumulation (C) can be partitioned between leaf area growth and leaf thickening and some were consumed in growth respiration; it was partitioned between expansive growth for water uptake and addition of new mass to the tissue (Weraduwage *et al.* 2015).

Smaller leaf area with lower production of aboveground dry weight depicted higher growth efficiency, compared to larger leaf area but lower production of aboveground dry weight such as T17, FH95, and HC95. Correlation also depicted a highly significantly negative relationship between leaf area and growth efficiency. A 1% increase in partitioning of leaf thickening with corresponding 1% decrease in partitioning to leaf area growth was found to lead to a 28% decrease in plant mass (Weraduwage *et al.* 2015). An insignificant different of fruit dry weight among the accessions (Table 4) was attributed to the fact that the axillary buds of the plant that turn into fruit were falling from the plant after ripening, which resulted in a considerable decrease of the fruit dry weight, thus affecting the end result during harvesting stage. This was reported by Scott and Cook (1995) and Rademacher (2015), in cases where the fruit was collected earlier than stem yield for seed production purposes. Meanwhile, the planting date, fertilization, and plant population were altered to maximize seed yields while limiting the vegetative growth plant.

The rate at which transpiration occurs refers to the amount of water lost by plants over a given time period. It is suggested that better intercellular concentration of carbon dioxide resulted in better E_L (Silva *et al.* 2013). V4383 possess higher WUE_i, which may be due to its higher A_{net} and lower G_s. In other words, increasing of G_s led to increasing the process of water vapour loss through stomata. V4202 meanwhile possesses the lowest WUE_{inst} due to its high E_L but low A_{net}. Water use efficiency (WUE) is an important indicator for leaf carbon and water fluxes at leaf level. It can be determined by WUE_{inst} and WUE_i that are expressed as the quotients of the diffusive fluxes of CO_2 into the leaf and water vapor out of the leaf during photosynthesis (Farquhar and Richards 1984). This means that V4202 releases a high amount of water during transpiration but low carbon assimilation occurs to produce energy; thus, the water use of the plant was not efficient. This was in agreement with Willis and Balasubramaniam (1968), who reported that E_L is sensitive to plant biomass, but further it is more sensitive to A_{net}.

The WUE_i of the control accession was much higher than Tahery *et al.* (2011) (11.3 μmol mol^{-1}). The function of G_s is to absorb CO_2 from the environment to be used for food synthesis, and release oxygen to the environment as the product from the food synthesis (Farquhar and Sharkey 1982). The increase in opening of stomata, however, may release more water from the leaves. This decreases the ratio of carbon assimilation to water loss. Thus plants with high A_{net} and low G_s provides better WUE_i towards plant growth and possess better control of stomatal function.

Willis and Balasubramaniam (1968) studied that the stomata of leaves may open in response to light, but close again fairly quickly as the stress increases. This condition may increase stress of photosensitive plants (such as *H. cannabinus*), therefore leading to early flowering and decreased yield. This can be seen in Fig. 1, which indicates that the stem height and stem diameter increment of T17 accession decreased considerably after two MAP. The parameter taken of WUE_{inst} and WUE_i also depicted that T17, T15, and V4383 accessions were tolerance to water deficit condition and response to light. The lower WUE_i

such as the control and HC2 accession meanwhile depicted that the accession need well-irrigation for better yield.

Fiber diameter and tensile modulus were significantly different ($p \leq 0.01$) among *H. cannabinus* accessions. The significance of fiber diameter and tensile strength among accessions agreed with Hossain *et al.* (2011). The fiber tensile strengths greater than 30 MPa were observed from fibers, which showed rough surfaces and fewer impurities. Meanwhile, the fiber tensile strengths less than 20 MPa had voids, impurities, and showed fiber damage on the surface. The tensile modulus in present study, however, was lower than the previous study by Abdul Khalil and Suraya (2011), as the sample taken at the bottom of the stalk while Rowel and Stout (1998) reported that kenaf fiber length increased from bottom to top.

Correlation

The relationship between stem dry weight and total aboveground dry weight were highest ($r = 0.848$) compared to the relationships between another morphological and yield characteristics with total aboveground dry weight (Table 8). This indicates that stem dry weight contributed to the greatest proportion of the total aboveground dry weight. This agreed with similar studies by De Andres *et al.* (2010) and Hossain *et al.* (2012), where the highest proportion (67.0%) of dry matter was found in the stem of *H. cannabinus* varieties. Heavier total aboveground dry weight directly produced heavier stem yields, which are crucial for *H. cannabinus* fiber production.

Fruit dry weight was found significantly associated with all morphological and yield characteristics, except for the root dry weight, leaf area, and growth efficiency. The results (Table 8) also indicate that fruit dry weight contributed to the second largest proportion in total aboveground dry weight, but it cannot be used as a determinant factor as for growth efficiency. Significant positive relationships were found between leaf area with stem height, stem diameter, stem dry weight, and total aboveground dry weight. This indicated that larger leaf area encourages higher photosynthesis rates, which is pertinent for plant growth performance (Clavijo-Herrera *et al.* 2018). Crowder and Chedda (1982) observed the stem proportion increase as leaf expansion due to an increase in the proportion of lignified structural tissue resulted from photosynthetic process. However, an insignificant relationship was observed between leaf area and leaf dry weight; the leaf area of *H. cannabinus* accessions from different origins (such as Malaysia, China, and Bangladesh) possess different C partitions in the leaf. This was partitioned between expansive growth for water uptake (such as V4383) and addition of new mass to the tissue (thicker leave such as T19) or both.

Increase in leaf area could explain the decreases in *H. cannabinus* growth efficiency. Weraduwage *et al.* (2015) explained that area-based photosynthesis shows a weak correlation with relative growth rate of plant mass. The differences in relative growth rate of plant mass between the accession is very sensitive to variations in parameters related to leaf area including leaf area per unit leaf mass and the proportion of total plant mass invested in leaf, or leaf mass ratio. The results confirmed the finding by Abdul-Hamid *et al.* (2009) that growth efficiency is low as a consequence of the higher reading in leaf area. The growth efficiency also found significantly ($p \leq 0.05$) weak negative relationships with stem diameter ($r = -0.158$) and stem dry weight ($r = -0.160$) (Table 6), which indicated that the growth efficiency progressively declined with age of the plant.

Increase in G_s resulted in increased A_{net} and a further increase in carboxylation efficiency. However, the decrease in G_s was not the only factor that decrease in A_{net}. The results confirmed the findings in previous studies (Pallas *et al.* 1966; Radin *et al.* 1988; Wilkinson and Davies 2002; Sapeta *et al.* 2013) that in conditions where the G_s is low while the Ci is high (such as in V4202 in present study), there are other factors (such as abscisic acid hormone from the root that minimized water losses due to transpiration) involved in controlling the photosynthesis process. Decreasing of G_s also could be more associated with decreases in E_L (Table 8) that responded to soil moisture content rather than leaf turgor. The finding was similar with Pallas *et al.* (1966), who found the greatest percentage of open stomata found during water sufficiency.

Increase in A_{net} indicates increased in G_s, E_L, carboxylation efficiency, and a decrease in Ci; the low Ci triggers the opening of stomata pores (Table 8). Radin *et al.* (1988) found a significant relationship between A_{net} and G_s in cotton (*Gossypium hirsutum* L.) canopy in the field. Even though the closure of stomata is an immediate response to water deficit in order to reduce water loss and increase the resistance to carbon dioxide diffusion, the carboxylation efficiency, however, only was affected after the stomatal closure had already completed. The present study also observed that a condition of high Ci present with low Gs and carboxylation efficiency (Table 5). Tominaga *et al.* (2018) reported there are uncertainties in calculating Ci when stomata close. The G_s is the determining factor for carbon assimilation, while Ci cannot be used to determine carbon assimilation in water-stress conditions (Da Veiga and Habermann 2013). A biphasic response was reported by Brodribb (1996) that initial stomatal control phase resulted in a substantial reduction in Ci as G_s decreased. As G_s reached a low level, a strong nonstomatal limitation phase was observed, causing Ci to increase as G_s approached a minimum level.

The significance of water to physiological responses of *H. cannabinus* is important, as the information could be used for genetic improvement regarding intrinsic site conditions. Enhanced A_{net} and WUE contribute to maintain plant growth for the majority of the accessions. The HC2 accession could be considered for breeding line selection, as it possesses the highest A_{net} and G_s, which contributed to a high amount of dry matter. Additionally, the V4383 accession could be considered for its high value of WUE_i, which might contribute to good adaptation to low water potential.

The lower tensile modulus of the IBFC, China accessions, except for FH952, might have been due to early growth decreases (third MAP) compared to another accessions where growth decrease at four MAP. Early growth decreases might be related to early maturity cycles, which led to fiber deterioration of IBFC, China accessions (except FH952) during harvest time (fourth month after planting). The fiber tensile strengths greater than 30 MPa were observed from fibers that showed rough surfaces, fewer impurities (cellulosic and non-cellulosic constituents), and no fiber damage. In contrast, the fiber tensile modulus less than 20 MPa had voids, impurities, and showed fiber damage on the surface. The HC95, V4202, and T15 accessions had lower tensile modulus with excessive fiber damages that could affect the fiber mechanical properties. In other words, these fiber accessions were more brittle than other accessions. The control, HC2, FH952, and V4383 accessions could be recommended for breeding line selection as they possessed higher tensile modulus with a rough fiber surface, in which is important for interface bonding of polymer and kenaf fiber (Mardin *et al.* 2016).

CONCLUSIONS

1. All measured morphological quantitative and yield characteristics were significantly different among accessions, except for stem dry weight, fruit dry weight, and total aboveground dry weight.
2. Stem diameter was positively and significantly correlated with all morphological and yield characteristics except for leaf dry weight and growth efficiency. Bigger stem diameter was an indicator of fiber yield in attempts to apply crossing and selection to improve performance
3. Photosynthesis rate was significantly positively correlated with stomatal conductance, transpiration rate, instantaneous water use efficiency, and carboxylation efficiency. High photosynthesis rate could be an indicator to interpret the pattern of genetic variation of plant assimilation rate and its relation with environmental and agronomic factors.
4. Fiber tensile modulus was found to be inversely correlated with fiber diameter.
5. The control, V4383, HC2, and FH952 accessions, can be regarded as a superior *H. cannabinus* resources possessing high fiber yield, fiber strength, and photosynthetic efficiency for breeding.

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