UNIVERSITI PUTRA MALAYSIA

IMPACT OF WATER STRESS AND CO2 ENRICHMENT ON GROWTH AND FIBER DEVELOPMENT OF KENAF (Hibiscus cannabinus L.)

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FP 2016 43

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By

AMIR MAHDI KHALATBARI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2016

DEDICATION

Dedicated with Love to

My Kind Father, Mohsen Khalatbari

and

My Beloved Mother, Azam Porsheikhani

and

My Supportive Brother, Amir Ali Khalatbari

For Their Endless Love, Support and Sacrifices

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

IMPACT OF WATER STRESS AND CO_2 ENRICHMENT ON GROWTH AND FIBER DEVELOPMENT OF KENAF (*Hibiscus cannabinus* L.)

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July 2015

Chairman: Associate Professor Hawa ZE Jaafar, PhD
Faculty: Agriculture

In this study, the effect of some environmental factors (different water treatments and CO_2 concentration) on morphological, physiological and histochemical characteristics of some kenaf varieties namely Fuhong (FH991, FH992 and FH952), V36, Kohn-Kaen60 (KK60) and TK were considered. Kenaf (*Hibiscus cannabinus* L.), a fast growing C3 plant native of tropical Africa, is being investigated as a new source of bioenergy as well as an industrial crop and has high potential to be used in Malaysia. Kenaf is considered as a great source of cellulose fiber for a wide range of paper products. Information on lignocellulose properties (lignin and cellulose) of these kenaf varieties as affected by environmental factors such as water stress, time of water stress imposition and CO_2 enrichment are still scarce and such data would be useful to provide information on fiber products containing high levels of lignocellulose attributes. A Randomized Complete Block Design (RCBD) experiment was conducted to determine performance and growth rate of varieties namely Fuhong (FH991, FH992 and FH952), V36, Kohn-Kaen60 (KK60) and TK during the seedling establishment. Seedling establishment is one of plant's growth phases in which some of the factors are precisely influential in final stage of growth and development for kenaf which is known as fiber product. The results showed that different varieties had significant effects on growth parameters such as shoot height and stem diameter which are important for producing higher fibre content and yield. Varieties FH991 and V36 obtained higher values of plant height (74.2 cm and 72 cm respectively) and stem diameter (5.53 mm and 5.50 mm) whereas variety KK60 recorded the lowest value for root parameters such as root average diameter value which was 0.62 mm. These three varieties were selected for further studies for their fiber yield and quality evaluation. A split plot experiment was arranged to determine effect of three different water treatments (100% ER; well watered, 50% ER; high water stress and 25%

ER; severe water stress) on morphological, physiological, fiber yield and lignocellulose quality of varieties FH991, V36 and KK60. The highest plant height belonged to variety FH991at 100% of ER and followed by variety V36 (242.67 cm and 230.66 cm respectively) whereas variety KK60 with water treatment of 25% of ER obtained the lowest average height of 190.67 cm at the end of experimental period. The highest net photosynthesis rate belonged to variety FH991 at 100% of ER with the value of 23.57 µmol m^{-2} s^{-1} at the end of experimental period. The lowest net photosynthesis rate 10.87 of µmol m^{-2} s^{-1} was recorded by variety V36 at 25% of ER. The highest fiber length fore bast was recorded by varieties FH991 and V36 (2.59 mm and 2.57 mm respectively) at 100% ER. The highest fiber length of core belonged to varieties V36 and FH991 with 100% ER recording the value of 0.95 mm and 0.93 mm respectively. The lowest fiber length for bast belonged to variety V36 at 25% ER with the value of 1.32 mm. The lowest fiber length for core was recorded by variety KK60 with the value of 0.48 mm. The optimum value of bast fiber yield was obtained by variety V36 with 100% ER water treatment (13.3 g plant^{-1}) followed by variety FH991 (12.96 g plant^{-1}) and KK60 (11.77 g plant^{-1}) respectively. The lowest value for bast fiber yield was recorded by variety V36 at 25% ER (4.98 g plant^{-1}). The highest core fiber yield of 21.72 g plant^{-1} belonged to variety FH991with 100% ER water treatment which was followed by variety V36 with value of 21.32 g plant^{-1}Variety V36 at 25% ER attained the lowest value of 8.44 g plant^{-1}. The evidence from this study elucidated that the fiber quality of for all three varieties decreased by increasing the severity of water stress from 100% ER to 25% ER. For the third experiment a split plot experiment was arranged to determine effect of three different times of water stress imposition at different growth stages namely daily watering (100% ER; well watered), water stress imposition 1 month after seedling establishment completion (1MAS) and finally water stress imposition at flowering stage (AFS) on morphological and histochemical attributes of varieties FH991, V36 and KK60. The highest value of net photosynthesis rate belonged to variety FH991 (23.33 µmol m^{-2}s^{-1}) and it was followed by varieties KK60 with value of 23.27 µmol m^{-2}s^{-1}and V36 at 21.63 µmol m^{-2}s^{-1}. The lowest net photosynthesis rate of 11.37 µmol m^{-2}s^{-1}belonged to variety KK60 subjected to water treatment of 1MAS. Considering the impact of different water treatments on fiber dimensions of these varieties, the highest bast and core fiber length of 2.59 mm and 0.91 mm was recorded when all varieties were under control water treatment (no stress). The lowest bast and core fiber length belonged to water treatment of 1MAS with value of 1.62 mm and 0.64 mm respectively. The highest bast holocellulose, α-cellulose and lignin belonged to varieties under control treatment (85.22%, 56.42% and 13.75% respectively). Core lignocellulose attributes attained highest percentages under control water treatment in which holocellulose recorded 83.17%, α-cellulose 46.85% and lignin 20.08%. The lowest bast and core lignocelluloses belonged to varieties under water stress treatment of 1MAS. Bast holocellulose of 80.67%, α-cellulose of 56.42 and lignin of 13.75% were recorded when plants were under water stress of 1MAS. Plants under water stress treatment of 1MAS had lowest core holocellulose of 78.63%, α-cellulose of 41.97% and lignin of 15.24. As an interesting result water stress imposition at flowering stage increased bast fiber length and core lumen diameter for selected varieties that could describe possible positive effect

of water stress imposition in this stage of growth on kenaf fiber dimension. For the last experiment varieties FH991, V36 and KK60 were exposed to 400 and 800 µmol mol^{-1} CO_2 in a split plot experiment. Results of the analysis of variance showed that different CO_2 concentrations (400 µmol mol^{-1} and 800 µmol mol^{-1}) had significant impact on morphological, physiological and histochemical properties of all three varieties for all traits measured; indicating enriched CO_2 level (800 µmol mol^{-1}) had a huge impact on measured traits. The highest bast fiber length was recorded by 800 µmol mol^{-1} CO_2 level with value of 3.10 mm whereas CO_2 level of 400 µmol mol^{-1} recorded bast fiber length of 2.68 mm. For core fiber attributes, the highest core fiber length belonged to elevated CO_2 level (800 µmol mol^{-1}) with value of 0.98 mm whereas ambient CO_2 level (400 µmol mol^{-1}) recorded core fiber length of 0.92 mm. The highest bast holocellulose, α-cellulose and lignin belonged to varieties under enriched CO_2 level of 800 µmol mol^{-1} (87.34%, 57.85% and 14.32% respectively). Core lignocellulose attributes attained highest percentages under elevated CO_2 level (800 µmol mol^{-1}) in which holocellulose recorded 84.24%, α-cellulose 47.52% and lignin 21.60%. The most obvious finding to emerge from this study is that CO_2 enriched kenaf plants exhibited the ability to synthesize higher fiber yield and lignocellulose properties which were not detected from kenaf grown under ambient CO_2 concentration. These results indicate that the fiber yield and histochemical attributes of these kenaf varieties can be enhanced by controlled environment production and CO_2 enrichment in top soil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

PENGARUH TEKANAN AIR DAN PERKAYAAN CO_2 TERHADAP PERTUMBUHAN DAN PEMBENTUKAN FIBER KENAF (*Hibiscus cannabinus* L.)

Oleh

AMIR MAHDI KHALATBARI

Julai 2015

Pengerusi: Prof. Madya Hawa ZE Jaafar, PhD
Fakulti: Pertanian

Kenaf (*Hibiscus cannabinus* L.), tumbuhan C3 yang cepat membesar berasal dari Afrika tropika, sedang disiasat sebagai sumber baru biotenaga dan juga sebagai tanaman industri berpotensi tinggi untuk digunakan di Malaysia. Kenaf dipertimbangkan sebagai sumber hebat fiber selulosa untuk produk kertas berskala besar. Satu kajian telah dilakukan untuk menilai dan membandingkan morfologi, fisiologi dan ciri-ciri histokimia enam variety kenaf terhadap perbezaan rawatan air dan tahap CO_2 di Serdang, Selangor, Malaysia. Objektif kajian ini secara keseluruhan adalah untuk menentukan keadaan optimum dari segi tekanan air dan kepekatan CO_2 untuk hasil dan kualiti fiber. Secara umum, semua varieti kenaf adalah lebih baik apabila disiram dengan secukupnya berbanding dengan kenaf yang diletakkan di bawah rawatan tekanan air berdasarkan kaedah penggantian evapotranspirasi atau perbezaan masa tekanan air dilaksanakan. Varieti FH991 dan V36 didapati menghasilkan panjang pucuk paling tinggi dan diameter batang yang merupakan ciri paling penting untuk penghasilan hasil. Nilai tertinggi untuk kadar bersih fotosintesis direkodkan oleh varieti FH991 dan V36 berikutan rawatan air kawalan (tiada tekanan) yang mana disebabkan konduksi stomata yang tinggi. Pengurangan adalah lebih parah dalam kes potensi air daun, membawa kepada stomata yang hampir tertutup, pengurangan pengambilan CO_2 dan menyebabkan aktiviti fotosintesis yang terhad untuk semua varieti di bawah rawatan tekanan air. FH991 dan V36 mencapai hasil kulit fiber, hasil pusat fiber, hasil keseluruhan fiber dan nilai lignoselulosa tertinggi yang mana lebih signifikan dari tumbuhan dibawah tekanan air. Keputusan analisis variant menunjukkan perbezaan kepekatan CO_2 (400 μmol mol^{-1} dan 800 μmol mol^{-1}) mempunyai kesan yang signifikan terhadap morfologi, fisiologi dan sifat histokimia kesemua tiga variety untuk semua ciri yang diukur; menunjukkan perkayaan tahap CO_2 (800 μmol mol^{-1}) mempunyai kesan yang besar terhadap ciri-ciri yang diukur. Tumbuhan yang terdedah

kepada perkayaan tahap CO_2 800 µmol mol^{-1} juga didapati merekodkan tinggi pokok dan biomas keseluruhan tertinggi. Di kalangan varieti, FH991 mempunyai ketinggian pokok, luas daun keseluruhan dan biomas keseluruhan paling tinggi yang mana memberikan hasil fiber keseluruhan tertinggi. Ciri fisiologi terutamanya kadar fotosintesis dan kadar transpirasi meningkat apabila tumbuhan didedahkan kepada perkayaan tahap CO_2 terutamanya FH991 dan ini kerana konduksi stomata yang tinggi yang dilakukan oleh tumbuhan dibawah kepekatan CO_2 yang tinggi iaitu 800 µmol mol.$_{-1}$. Varieti berbeza tidak mempunyai kesan signifikan terhadap ciri fisiologi dan tiada kesan interaksi yang signifikan antara varieti dan tahap perkayaan CO_2 terhadap ciri fisiologi. Apabila tumbuhan terlibat dengan perkayaan tahap CO_2 800 µmol mol^{-1}, varieti FH991 dan V36 mempunyai hasil kulit dan pusat fiber yang tertinggi dan menunjukkan nilai panjang fiber dan ciri lignoselulosa yang lebih tinggi terutamanya holoselulosa dan α-selulosa yang mana penting untuk kualiti fiber yang tinggi sebagai pertimbangan untuk pengeluaran kertas. Mengambil kira keseluruhannya, kajian ini memberikan maklumat bernilai untuk pembentukan fiber, ciri-ciri histokimia varieti kenaf tempatan yang mana penting untuk usaha pembangunan di masa hadapan.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Associate Professor Dr. Hawa ZE Jaafar, chairman of my supervisory committee, for her dedicated efforts, support, invaluable advice and intellectual guidance during the accomplishment of this research work. I would also like to thank my supervisory committee members, Associate Professor Dr. Hazandy Abdul Hamid and Dr. Mohd. Ridzwan Abd Halim for their guidance, assistance, encouragements and constructive comments throughout the period of this study. I greatly appreciate all the help and support provided by the supervisory committee during my study in Malaysia.

Special thanks go to Universiti Putra Malaysia (UPM) for their contribution for this project number of 5523867 FRGS (Fundamental Research Grant Scheme).

I am very grateful to Mr. Rodhi Ahmed and all staff/officers of TPU and Ladang 2, Universiti Putra Malaysia for their help during my field work. I am also grateful to the laboratory technicians of the Department of Crop Science, Universiti Putra Malaysia.

My sincere thanks and appreciations also go to my friends and fellow students especially Dr. Ali Ghasemzadeh and Dr. Ehsan Karimi for their help, support and encouragements during the period of my study.

My deepest gratitude goes to my father Mohsen, my mother Azam and my brother Ali for their help and continuous moral support throughout my study.

I certify that a Thesis Examination Committee has met on 8 July 2015 to conduct the final examination of Amir Mahdi Khalatbari on his thesis entitled "Impact of Water Stress and Co_2 Enrichment on Growth and Fiber Development of Kenaf (*Hibiscus cannabinus* L.)" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The committee recommends that the student be awarded the degree of Doctor of Philosophy.

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Signature: ______________________________

Name of Member of Supervisory Committee: **Associate Professor Dr. Hazandy Abdul Hamid**

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Name of Member of Supervisory Committee: **Associate Professor Dr. Mohd Ridzwan A. Halim**

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LIST OF ABBREVIATIONS

ºC	Degree Celsius
°N	North
°S	South
AFS	At Flowering Stage
ANOVA	Analysis of variance
B.C.	Before Christ
CEC	Cation exchange capacity
CGR	Cumulative Growth Rate
Chl	Chlorophyll
Chl F	Chlorophyll fluorescence
cm	Centimetre
CO_2	Carbon Dioxide
C.V	Coefficient of variation
d.f	Degree of freedom
DNMRT	Duncan New Multiple Range Test
dNTP	2'-Deoxynucleoside 5'- triphosphate
ER	Evapotranspiration Replacement
EDTA	Ethylenediaminetetraacetic acid
FC	Field Capacity
FAO	Food and Agriculture Organization of the United Nations
Fv/fm	Fluorescence variable/ fluorescence maximum
g	Gram
ha	Hectare
hr	Hour
K	Potassium
kPa	Kilo Pascal
m^2	Square meter
M^3	Cubic meter
LRWC	Leaf Relative Water Content
LSD	Least significant difference
LWP	Leaf Water Potential
MAS	Month After Seedling
MPa	Mega Pascal
MS_e	Mean square of error
µg	Microgram
µl	Microlitre
µm	Micrometer
µM	Micromolar
mm	Millimeter
mmol	Millimol
µmol	Micromol
N	Nitrogen
NS	Not Significant
P	Phosphorus
pH	Potential of Hydrogen
ppm	Parts per million
R	Correlation coefficient
RCBD	Randomized complete block design
RWC	Relative Water Content

S	Second(s)
SAS	Statistical Analysis System
S.E.	Standard Error
S.O. V	Source of varience
SPAD	Special Products Analysis Division
St.Dev.	Standard deviation
TBE	Tris-borate/EDTA
UV	Ultraviolet
V	Volt
v/v	Volume per volume
WP	Water Potential
WUE	Water Use Efficiency

CHAPTER 1

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L., Malvaceae) is a warm-season annual crop, with a C_3 – photosynthetic pathway, achieving high biomass yields. Although kenaf is a C_3 crop and uses solar radiation, CO_2, water and nitrogen more efficiently than C_4 crops (Danalatos et al., 2005). In the early 1970s, kenaf was introduced to Malaysia and then identified as a potential alternative and cheaper source of fibrous material (Azizi et al., 2010). It has enormous potential to be Malaysia's next industrial crop since it can be used in different types of products with commercial value (Abdul Khalil et al., 2010). The National Kenaf Research and Development Program has been established for research and future development of kenaf–based industry, whereas the government has considered and allocated RM 12 million under the 9th Malaysia Plan (2006-2010) for this purpose (Edeerozey et al., 2007). Kenaf is a demanding crop and is less sensitive to weather and environmental conditions compared to tobacco as kenaf is known to be a tolerant plant to water stress. This has encouraged farmers to replace tobacco with kenaf (Azizi et al., 2010).

Emission of carbon dioxide (CO_2) has become a major concern in many developed and developing countries in order to deal with global warming issues. Therefore, choosing proper strategies to reduce the CO_2 concentration would be achieved by executing carbon capture method. Kenaf as a green source is a great CO_2 sequester which could be great choice to attain this target. Kenaf is an environmental–friendly industrial organic material which Kyoto Protocol has recognized it as a plant with the ability to combat global warming issues. It was reported by Lam et al., (2003) that growing global warming and its environmental concern have led to increasing interest in kenaf as a source of cellulosic fiber for its high CO_2 fixation ability. The use of kenaf as an alternative raw material to wood will protect some forest resources from further deforestation and results in environmental stabilities. Carbon dioxide enrichment entails increasing carbon dioxide concentration to 2 to 4 times higher than the normal atmospheric levels, to between 800-1500 µmol m^{-2} s^{-1}, in an enclosed space (Wooodward, 2002). Based on this scientific definition two CO_2 concentration of 400 µmol m^{-2} s^{-1} as an ambient level and 800 µmol m^{-2} s^{-1} were introduced in this study to kenaf varieties to evaluate the effect of CO_2 on fiber yield and quality. CO_2 enrichment has been shown to enhance growth rate and plant yields (Tisserat *et al*., 1997; Wang *et al*., 2003; Onoda *et al*., 2007) and the increase of CO_2 concentration in the atmosphere is also well documented (Wooodward, 2002). Reported positive effects of enriched CO_2 levels on plant growth encouraged optimism among scientists for future agricultural production (Jaafar, 2006).

As an abiotic stress, water stress is the one of the most serious limitations in agriculture thus; water tolerance performed by any plant is really immensely important (Shao et al., 2008). This type of tolerance involves the changes and differences in biochemistry, physiological responses like leaf gas exchange of plant and plant water relation (Guerfel et al., 2009). Sufficient amount of water should be supplied for plants in order to maintain their cells in good conditions at the early stage of development in order to produce new tissues and cells progressively (Neumann, 2008).

1.1 Justification

The Malaysian pulp and paper industry identified kenaf fiber as a stable source of non-wood materials and a suitable alternative for wood fiber (Roda and Rathi, 2006). However, introduction and determination of specific varieties from local and foreign sources with fiber production is still in its early stages in Malaysia. In Malaysia Development of kenaf industry has both weak and strong noticeable aspects. First of all, Malaysia as a tropical country has the suitable climate in which kenaf cultivation can be very successful. The government and Research Departments of Malaysia are strongly interested in the cultivation of this plan due to prospective market of fiber production in the region. However, lack of enough cultivation knowledge is main setback in developing kenaf industry in Malaysia.

No articles have reported data on the physiology and histochemical properties of kenaf to be grown in top soil (SERDANG series) in Malaysia. Kenaf was reported to produce higher stem production on fertile soils as oppose on BRIS soil. It was concluded after a number of trials of Kenaf cultivation on BRIS that Kenaf is adapted to a wide range of soil types, but performs best on the fine to medium textured clay or loamy, well drained, fertile soils like Serdang series. The Serdang Series as a well-drained soil to over 100 cm depth can be considered. It has good permeability. A large variety of crops can be grown on these soils. These include crops such as oil palm, rubber, fruit trees etc. (Panton, 1954). So it seems valuable to determine the performance of this plant in this type of soil under controlled condition especially for conducting CO_2 enrichment experiment and different water treatments.

The production of fiber may be much affected by the environmental factors which can dwindle as climate scenario changes in future. In order to bring proper insight, examination and determination of physiological and histochemical aspects of Malaysian kenaf cultivars under condition of changing climate (such as different water treatments and CO_2 levels) seem valuable. Crop cultivation modern high technology in controlled environmental system (CES) structures such as glasshouses, greenhouse and rain shelters have many advantages (Jaafar, 2006). The findings were, however, not conclusive and need further research to establish the environmental conditions that would enhance growth and carbon assimilation for increased yield and sustainable production of quality fiber for kenaf under controlled environment system. One of the major driving forces of photosynthesis is CO_2. The greenhouse industry has taken advantage by manipulating this factor for the benefit of crops grown in controlled conditions for extended periods. In order to have proper control and to observe the effect of specific water treatments and different CO_2 concentrations on kenaf growth, fiber yield and quality, experiments had to be done in glasshouse to discard all other undesirable environmental factors.

One of the means to increase different aspects of production is to speed up the growth process. This could be plausible by using carbon dioxide (CO_2) with different levels from seedling stage on. Increase in carbon gain is justified by augment of total plant dry biomass considering enhancement of photosynthesis with extra supplies of carbon dioxide. Despite the enhancement of photosynthesis, growth and production would be facilitated with reduction of transpiration that results in higher water use efficiency (Hsiao and Jackson, 1999). Plant growth and photosynthesis stimulation have been demonstrated when level of CO_2 is higher than ambient (Coleman and sage, 2001). Despite its great potential for multiple uses (paper, construction materials, and energy), data on assimilation rate as affected by environmental factors such as water stress, time of water stress imposition and CO_2 enrichment effect on kenaf are scarce. Knowledge on kenaf carbon balance, as a response of assimilation and respiration rate to environmental conditions such as CO_2 concentration, is fundamental for understanding and assessing kenaf growth and productivity.

1.2 Hypothesis

As little study has been done to demonstrate that kenaf may continue growth under water stress condition and time of stress imposition, the present study is about understanding the impact of water stress and CO_2 enrichment on growth and fiber development of kenaf. There are possibilities of positive effect of CO_2 enrichment and specific water treatment (as a trigger) on fiber quality of kenaf especially before flowering time. The reduction in transpiration rate in plants under water deficit may also be attributed to morphological changes such as increased cell wall thickness and cell wall lignifications which could bring higher fiber quality (Netondo, 1999).

1.3 General Objective

To determine performance of specific varieties from local and foreign sources with fiber production under some influential environmental factors such as water stress and CO2 enrichment.

1.4 Specific Objectives

1) To evaluate the performance of kenaf varieties on top soil
2) To determine the best water treatment and the most effective time of stress imposition for higher fiber yield and quality.
3) To examine impact of CO2 enrichment on kenaf yield, especially on bast and core fiber biophysical properties.
4) To identify lignocellulose properties of each selected varieties under water treatments, the time of stress imposition and CO2 enrichment.
5) To determine the most potential productive varieties under CO2 enrichment.

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