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# EFFECTS OF KENAF (*Hibiscus cannabinus L.*) MUTANT LINES UNDER WATER STRESS CONDITION USING $^{15}$N AND $^{13}$C STABLE ISOTOPES

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## *ABSTRACT*

*Kenaf is a fibrous plant which has potential in various industrial applications. The effects of water stress on growth and water relations of several Kenaf (Hibiscus cannabinus L.) varieties were investigated. The control variety, V36 and two of its mutant lines were used in the study. Three watering regimes namely field capacity (FC), 45% of field capacity (45FC) and 75% of field capacity (75FC) were imposed on the plants. Each watering treatment and variety was replicated three times in a completely randomized design. Nitrogen, phosphorus and potassium fertilizer were applied onto plants at two split applications. Soil water stress significantly reduced vegetative growth as measured by plant height, collar diameter growth, leaf development, pod numbers and stem yield. Highest nitrogen use efficiency (NUE) and water use efficiency (WUE) were obtained through 45FC water treatment. The stem yield of S3 mutant population under FC treatment was the highest among other varieties. The S3 population is significantly used nitrogen and water efficiently than other populations. FC water treatment is suggested as the best application to grow kenaf, and the S3 mutant line is recommended to grow under moderate water stress condition if necessary.*

**Keywords**: Carbon, fiber, kenaf, nitrogen, stable isotopes, water stress

## INTRODUCTION

*Hibiscus cannabinus* (Kenaf) is a single-stemmed, annual to perennial plant growing around 1.5 – 4.5 meters tall and the stems can become more or less woody and persistent (Ramesh, 2016). The origin of this plant is from Africa and it has been identified for almost 4000 years ago. This kenaf plant stems contain two very useful types of fiber which are on the outer and the inner part. The outer fibers known as skin fibers or bast and contain approximately 40% of dry stalks weight. Kenaf has a huge potential for the biocomposite industry in our country. It is not only produces high quality fibers, but also can accommodate some other manufacturing sectors such as mattresses and furniture, as well as acting as an amplifier fiber of the plastic component industry besides being a source of animal feed (Zainuddin, 2015). Besides, chemically modified kenaf fiber can be used as a sorbent material towards the purification of waste water, smart textiles, electrostatic discharge protection, and reinforcement in composites (Osman et al., 2017; Razak et al., 2017). Therefore, the high commercial value of kenaf makes it as an alternative to other crops to be cultivated.

The water requirements for kenaf cultivation is 7 to 15 mm per day for seed germination and initial crop establishment (Mat Daham et al., 2015). Adequate irrigation or rainfall during the growing season could maximize the growth and yield. Kenaf can withstand drought conditions, yet the quality of the fiber and length of roots will be slightly affected (Basri et al., 2014). Mostly, kenaf cultivation for seed production is in the northern area of Malaysia because of longer dry season (Mat Daham et al., 2015). Previously, kenaf was planted on BRIS soil to replace tobacco through the contracted farming method in the eastern coast of Malaysia. This type of soil has the low water retention capacity due to the sandy texture in nature (Basri et al., 2014). The practices kenaf grown in arid regions and the use of BRIS soil, increase the probability of water stress during growth that limits crop productivity. The adverse effects of water stress in plants are leafing water potential declines, leading to stomatal closure, decline in $CO_2$ uptake and limitations in photosynthetic activities, wilting, and dramatic impairment of many metabolic functions (Lisar et al., 2012).

The use of stable isotope variation in plant research is continuously growing since the past two decades (Yousfi et al., 2013). This trend will continue as scientists realize that stable isotopes can serve as a time-integrated indicators of how plants interact with and respond to their abiotic and biotic environments (Dawson et al. 2002). In that context, the analysis of the natural abundances of the stable isotopes in plants is very useful for crop physiology studies. The stable isotopes of carbon ($^{12}C$, $^{13}C$), oxygen ($^{16}O$, $^{18}O$) and nitrogen ($^{14}N$, $^{15}N$) are commonly being used in various agricultural fields. This study focuses on the utilization of nitrogen and carbon isotopes. The carbon isotope fractionation reflects limitations on photosynthetic efficiency imposed by the various diffusional and chemical components of $CO_2$ uptake (O'Leary, 1988). The use of $^{15}N$ (nitrogen isotope) as a tracer is the most dynamic tool to differentiate between the fates of a particular N (nitrogen) source and background soil N (Chen et al., 2016).

Selection of a suitable genotype with high growth rates and biomass production is essential for successful commercial cultivation of kenaf. There are six cultivars that are recommended as commercially suitable genotypes for the Malaysian tropical climate; Everglade71, HC2, HC78, Thai kenaf, V36, and V133 (Basri et al., 2014). Results from previous studies have shown that photoperiod insensitive and late-flowering cultivars were suitable for the tropical climate of Malaysia (Basri et al., 2014). The highest yield to be reported was from the accession V36 with 9.68 tonnes per hectare (Basri et al., 2014). Therefore, V36 kenaf variety has been selected to be irradiated for development of new mutants which possible to adapt in a wider range of conditions and environmental factors (Mohd Zulmadi et al., 2017). This study was carried out to characterize and quantify the growth response of kenaf plants (the control and mutants) under water stress conditions using agronomical parameters and stable isotope analysis.

## MATERIALS AND METHODS

### Plant Materials

A kenaf (*Hibiscus cannabinus* L.) variety, V36 (S1) (provided by National Kenaf and Tobacco Board (LKTN)) and its mutant lines S2 and S3 were used in the study. These mutant lines are derived from previous mutation breeding work. Approximately, 450 seeds represented three replications of each variety were sown on jiffy growing media. The two weeks old seedlings were transplanted into the trough (1 m x 3 m) filled with topsoil. Plants were allowed to acclimate for 4 months in a glass-house with a 12 hour photoperiod, 32 ± 4 °C day temperature and 28 ± 4 °C night temperature.

## Fertilizer Application

To reduce nutrient deficiency as a limiting factor, the plants were fertilized with Nitrogen (N), Phosphorus (P) and Kalium (K) compounds. Nitrogen fertilizer with the rate of 90 kg N per hectare was applied using two split applications. The first 30 kg N was applied at 14 days after transplanting (DAT) and the remaining 60 kg N at 40 DAT. Basal application of phosphorus (60 kg P/ha) and potassium (60 kg K/ha) fertilizers were also applied on the kenaf.

## Water Stress Conditions

Three different watering treatments were applied in this study were field capacity water condition (FC), 45% of field capacity (45FC) and 75% of field capacity (75FC) representing the control, moderate stress and severe stress for FC, 45FC and 75FC respectively. The choice of watering treatment was based on current weather during the study. Each watering treatment was replicated three times in a completely randomized design. Water retention curve analysis was performed using the pressure plate method (Figure 1). The curve showed that the FC, 45FC and 75FC were adjusted at 10 (26% soil moisture (SM)), 30 (18% SM) and 100 kPa (13% SM) respectively. This process was done using watermark meter, (Irrometer, California) equipment. For FC treatment, the plants received water every day, for 45FC treatment, the plants were watered at a 3 day intervals, whilst for 75FC treatment, the plants were watered at 1-week interval. The plants were harvested at maturity stage approximately after four months of planting.

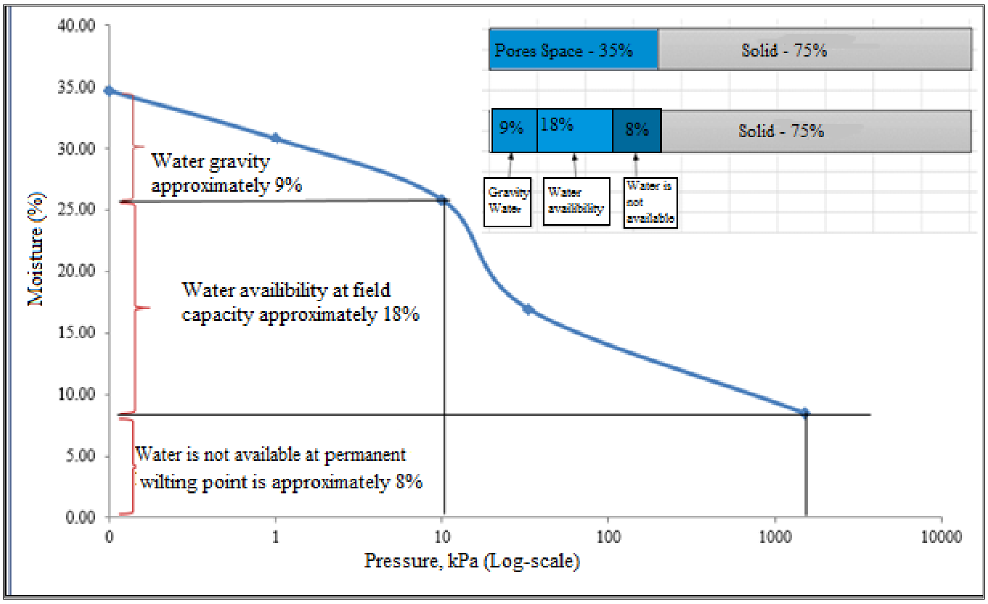


Figure 1: Water retention curve analysis FC at 10 kPa (26% SM), 45FC at 30 kPa (18% SM) and 75FC at 100 kPa (13% SM).

## Measurements

### Plant Growth

In each replicate, three plants per variety and treatment were harvested at the end of the experiment. Total plant biomass were weighed to obtain the overall fresh weight. Morphological data, including stem diameter, plant height, collar diameter growth, leaf development, pod numbers and plant yield were recorded in the form of mean value for further analysis. The plant vegetative parts were separated and the fresh weight of each was measured. Stem, leaves, shoots and pods were oven-dried at 70°C until constant weights were obtained. The dry weight of each component was determined and the parts were finely milled to reduce the particle size and to ensure the homogeneity of the samples.

## Nitrogen Use Efficiency (NUE)

The Kjeldahl method was used to determine the N content of kenaf samples. This method was divided into three steps; (i) Digestion – Decomposition of N in plant samples using a concentrated acid solution. It was performed by boiling a homogeneous sample in concentrated sulphuric acid to form an ammonium sulphate solution; (ii) Distillation – Adding an excess base to the acid digestion mixture to convert $NH_4^+$ to $NH_3^+$, followed by boiling and condensation of the $NH_3$ gas in a receiving solution and (iii) Titration method to quantify the amount of ammonia in the receiving solution (IAEA, 2001).

$^{15}N$ isotope in samples is determined by using an analytical instrument which is emission spectrometer NOI 7 C, (FAN, Germany). For the analysis, samples were first concentrated in liquid forms. Then, approximately 25 µl of the concentrated plant samples were inserted into a glass microtube and placed in the emission spectrometer chemical compartment. The analyzer is comprised of a data acquisition unit which stores the data and automatically converts the measured value to the true value (Ahmad Nazrul et al., 2017).

The formula to calculate the NUE is shown below:

$$^{15}N\ \text{grain} = \frac{\text{grain yield (kg per ha) x total N (\%) of grain}}{100} \qquad (1)$$

% Nitrogen derived from fertilizer (*Ndff*) = $$\%Ndff = \frac{^{15}N_{grain}}{^{15}N_{Fertilizer}} \times 100 \qquad (2)$$

$$Ndff = \%\,Ndff \times N\ taken\ up\ by\ crop \qquad (3)$$

$$\text{Nitrogen Use Efficiency (NUE)} = \text{FNUE} = \frac{Ndff}{\text{Total fertilizer N}_{\text{applied}}} \times 100 \qquad (4)$$

## Plant Water Use Efficiency (WUE) and Carbon Isotope Discrimination

Plant water uptake over the four-month growing period was measured by using water meters. It was installed with the irrigation system for assessment of WUE. WUE was determined as follows:

$$\text{WUE } (gL^{-1}) = \text{(Biomass yield) / (Total water used)} \qquad (5)$$

For carbon $^{13}C$ isotope discrimination ($\Delta^{13}C$), three young leaves per variety and treatment from different plants, developed after the outset of stress treatment, were sampled at the end of the study. The dried samples were homogenized by milling process and each was analyzed for $^{13}C$ content by using Elemental Analyzer-Isotope Mass Spectrometer (EA-IRMS), Sercon, UK. $\delta^{13}C$ content was presented in $\delta^{13}C$ ‰ units using the International Pee Dee Belemnite and the formula is shown below (Dercon et al., 2006):

$$\delta^{13}C\ (‰) = [(R_{sample}) / (R_{standard} - 1)] \times 1000 \qquad (6)$$

where R = ratio of $^{13}C/^{12}C$.

The $\delta^{13}C$ content of the plant material is associated to $^{13}C$ isotope discrimination ($\Delta^{13}C$) and the equation as follows:

$$\Delta^{13}C\ (‰) = [(\delta a - \delta p)/(1 + \delta p)] \times 100 \qquad (7)$$

$\delta a = \delta^{13}C$ value of air (-8 ‰)
$\delta p = \delta^{13}C$ value of leaf samples.

## Statistical Analysis

Data were analyzed using one-way ANOVA followed by Duncan’s test using Social Science (SPSS) version 21 and presented as mean ± SEM, where $p < 0.05$ was considered as significant.

## RESULTS

### Physical Growth

#### *Different water treatments*

The stem diameter was measured at three different stem parts; (i) Above the soil (bottom part); (ii) middle and (iii) top. The highest stem diameter on bottom part was obtained through FC water treatment with an average diameter of 14.64 cm. Similar to bottom part, the highest middle stem diameter was also obtained through FC water condition (10.13 cm). For the top part, the highest stem diameter measurement was obtained through 45FC water treatment (5.62 cm). FC treatment also generated plants with longer leaf length (13.51 cm in average) as compared to other treatments (45FC and 75FC). In terms of leaf width, plants treated with FC water treatment also showed wider leaves in general as compared to other water treatment. The average leaf width for FC plants was 9.49 cm, followed by 45FC (9.08 cm) and 75FC (6.05 cm). The highest plant height was obtained through FC water treatment with an average diameter of 271.06 cm. Besides, FC treatment also generated plants with higher pod numbers (30.83 in average) as compared to other watering treatments. The highest yield for the kenaf varieties was obtained through FC treatment. The calculated average yield in tonne per hectare (t/ha) is approximately 11.6 t/ha. Table 1 shows average plant growth observed for each water treatment.

Table 1: Average plant growth under different water treatments

| **Water Treatment** | **Stem Diameter (cm)** | | | **Leaf (cm)** | | **Plant Height (cm)** | **Pod Numbers** | **Plant Yield (t/ha)** |
|---|---|---|---|---|---|---|---|---|
| | **Bottom** | **Middle** | **Top** | **Length** | **Width** | | | |
| FC | 14.64 ± 2.22 | 10.13 ± 1.5 | 5.59 ± 0.69 | 13.51 ± 0.92 | 9.49 ± 0.5 | 271.06 ± 28.21 | 30.83 ± 9.36 | 11.6 ± 1.97 |
| 45FC | 11.43 ± 3.08 | 8.94 ± 2.37 | 5.62 ± 1.74 | 12.24 ± 2.83 | 9.08 ± 1.97 | 234.67 ± 45.92 | 28.72 ± 12.46 | 8.55 ± 1.99 |
| 75FC | 9.97 ± 0.73 | 7.35 ± 0.68 | 4.78 ± 0.66 | 8.69 ± 1.35 | 6.05 ± 1.29 | 200.17 ± 24.24 | 11.28 ± 4.19 | 4.07 ± 1.29 |

Figure 2 shows the graph for average stem diameter in different water treatment. The bottom stem diameter value of FC water treatment was the highest ($p < 0.05$) as compared to other water treatments. Figure 3 shows the graphs for average leaf length, leaf width, plant height and pod numbers in different water conditions. The 75FC water treatment resulted in significantly lower ($p < 0.05$) leaf length, leaf width and pod numbers as compared to FC and 45FC.

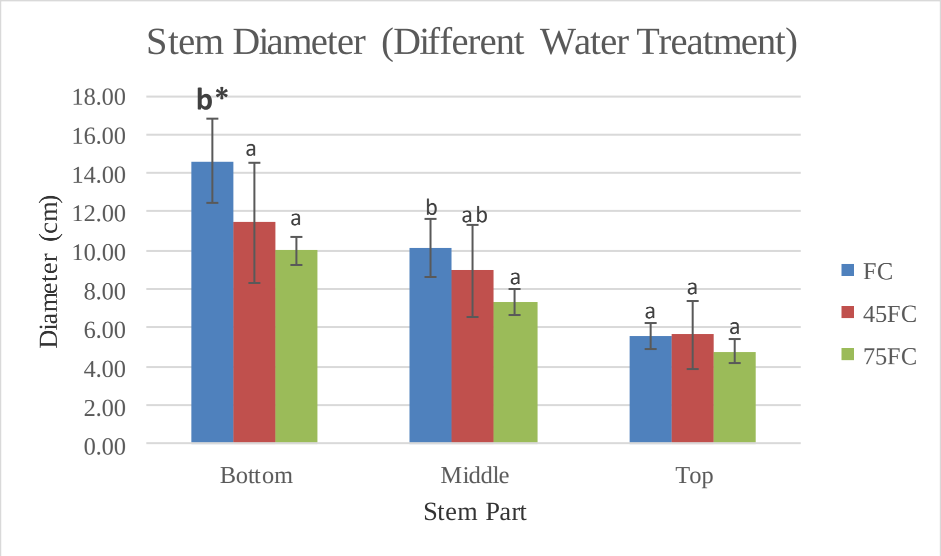


Figure 2: Average plant stem diameter in different water treatments.
**b*** indicates significant difference compared to other water treatments

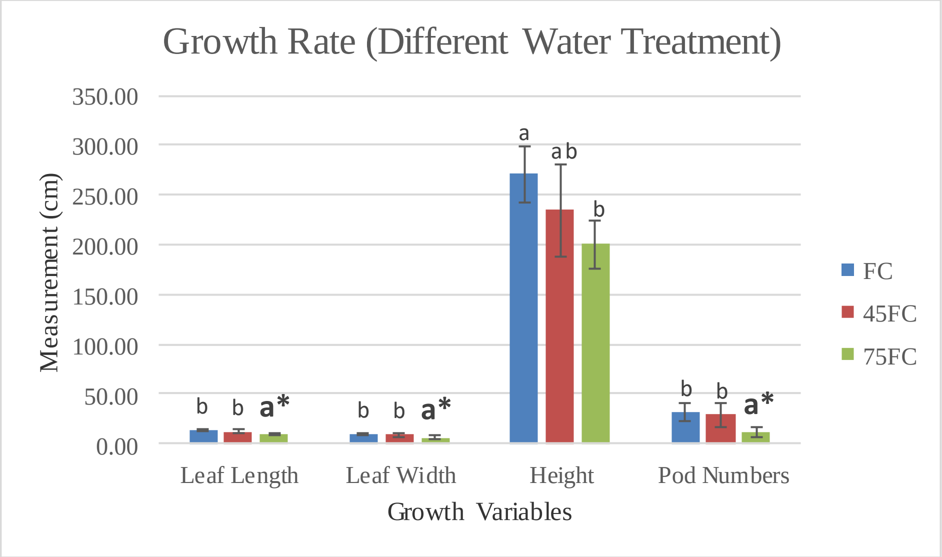


Figure 3: Average plant leaf length, leaf width, plant height and pod numbers in different water treatments. **a*** indicates significant difference ($p < 0.05$) compared to other water treatments

Figure 4 shows the average yield in different water treatment. The highest yield was recorded under FC treatment and the lowest yield was identified under 75FC water treatment ($p < 0.05$).

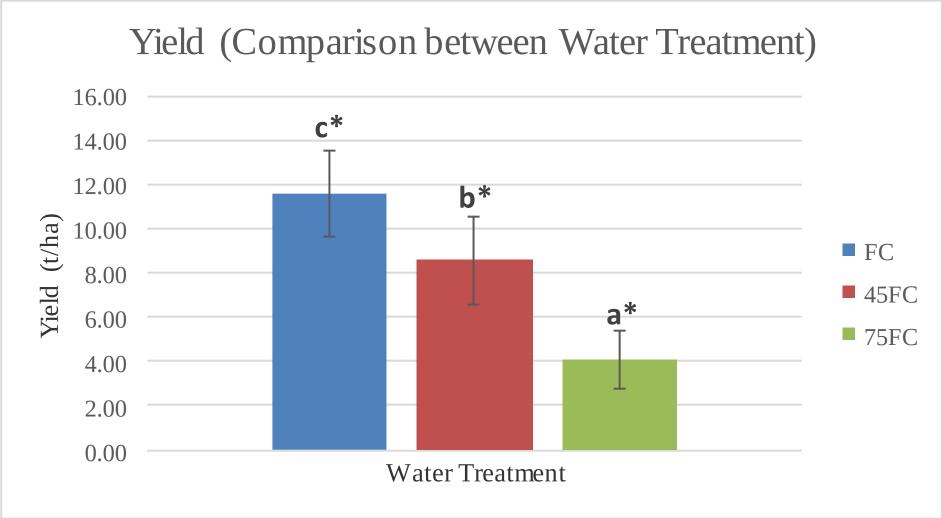


Figure 4: Average plant yield in different water treatments. a*, b* and c* indicate significant differences ($p < 0.05$) compared to other water treatments

***Variety Evaluation***

Individually, the S2 mutant population has the highest bottom stem diameter (14.95 cm) as compared to other varieties when treated under FC water condition. However, the highest middle stem diameter was S3 mutant population under the FC condition (10.45 cm). S2 mutant population has the highest top stem diameter through 45FC water treatment. For leaf length, the longest was observed in S3 mutant line (13.89 cm) under FC treatment. S1 and S3 varieties with the value of 9.56 cm have the widest width when treated under FC. S1 (FC), S2 (45FC) and S3 (FC) attained the highest mean values of 284.33 cm, 35.50 pods and 12.01 t/ha for plant height, pod numbers and plant yield respectively. Table 2 details out average plant growth of each variety population under different water stress treatments. Lowest results for bottom, middle and top stem diameter with the mean values of 9.61, 7.21 and 4.61 cm, respectively, were observed in S1 and S2 varieties under 75FC treatment. S1 population treated under 75FC attained the lowest leaf length and width with the values of 7.58 and 5.58 cm respectively. 75FC treatment in S1 and S2 varieties, causing lower average values of plant height (190.17cm), pod numbers (8.83) and plant yield (3.67 t/ha) as well.

Table 2: Average plant growth for different kenaf varieties under different water treatments

| **Water Treatment** | **Kenaf Variety** | **Stem Diameter (cm)** | | | **Leaf (cm)** | | **Plant Height (cm)** | **Pod Numbers** | **Plant Yield (t/ha)** |
|---|---|---|---|---|---|---|---|---|---|
| | | **Bottom** | **Middle** | **Top** | **Length** | **Width** | | | |
| FC | S1 | 14.38 ± 1.68 | 10.13 ± 1.83 | 5.10 ± 0.66 | 13.48 ± 0.74 | 9.56 ± 0.81 | 284.33 ± 43.76 | 34.50 ± 12.03 | 11.78± 2.96 |
| | S2 | 14.95 ± 4.04 | 9.8 ± 2.18 | 5.66 ± 0.84 | 13.17 ± 1.1 | 9.36 ± 0.13 | 260.17 ± 20.53 | 27.83 ± 10.49 | 11.01± 1.98 |
| | S3 | 14.59 ± 0.49 | 10.45 ± 0.75 | 6.03 ± 0.32 | 13.89 ± 1.12 | 9.56 ± 0.54 | 268.67 ± 19.90 | 30.17 ± 7.82 | 12.01± 1.39 |
| 45FC | S1 | 13.02 ± 4.97 | 9.93 ± 3.89 | 5.04 ± 1.33 | 12.69 ± 4.30 | 9.31 ± 2.63 | 219.33 ± 70.39 | 21.00 ± 14.40 | 8.18 ± 2.42 |
| | S2 | 10.41 ± 2.49 | 7.89 ± 1.48 | 6.79 ± 1.13 | 11.75 ± 3.18 | 8.58 ± 2.17 | 234.00 ± 51.88 | 35.50 ± 15.21 | 8.39 ± 2.47 |
| | S3 | 10.86 ± 1.07 | 8.99 ± 1.42 | 5.02 ± 2.44 | 12.28 ± 1.65 | 9.36 ± 1.84 | 250.67 ± 7.11 | 29.67 ± 4.75 | 9.09 ± 1.79 |
| 75FC | S1 | 10.24 ± 0.24 | 7.35 ± 0.48 | 4.61 ± 0.53 | 7.58 ± 1.45 | 5.58 ± 1.88 | 204.50 ± 29.55 | 12.00 ± 4.82 | 3.67 ± 0.66 |
| | S2 | 9.61 ± 0.94 | 7.21 ± 1.24 | 4.91 ± 0.73 | 9.22 ± 1.05 | 6.31 ± 0.97 | 190.17 ± 27.80 | 8.83 ± 5.25 | 4.56 ± 2.17 |
| | S3 | 10.07 ±0.95 | 7.50 ±0.20 | 4.83 ±0.93 | 9.25 ±1.18 | 6.25 ±1.31 | 205.83 ±21.88 | 13.00 ±2.29 | 3.98 ±0.93 |

Figure 5 shows the average stem diameter value for each individual variety population in different water condition. There was no significant difference ($p > 0.05$) in stem diameter among the plant varieties in FC (a), 45FC (b) and 75FC (c). S3 variety under 75FC had significantly lower ($p < 0.05$) leaf length (a), leaf width (b), plant height (c) and pod numbers (d) as compared to S3 variety under FC and 45FC water condition (Figure 6). Lower plant yield was recorded ($p < 0.05$) for S1 variety and S3 mutant population under 75FC as compared to S1 and S3 under FC and 45FC water condition (Figure 7).

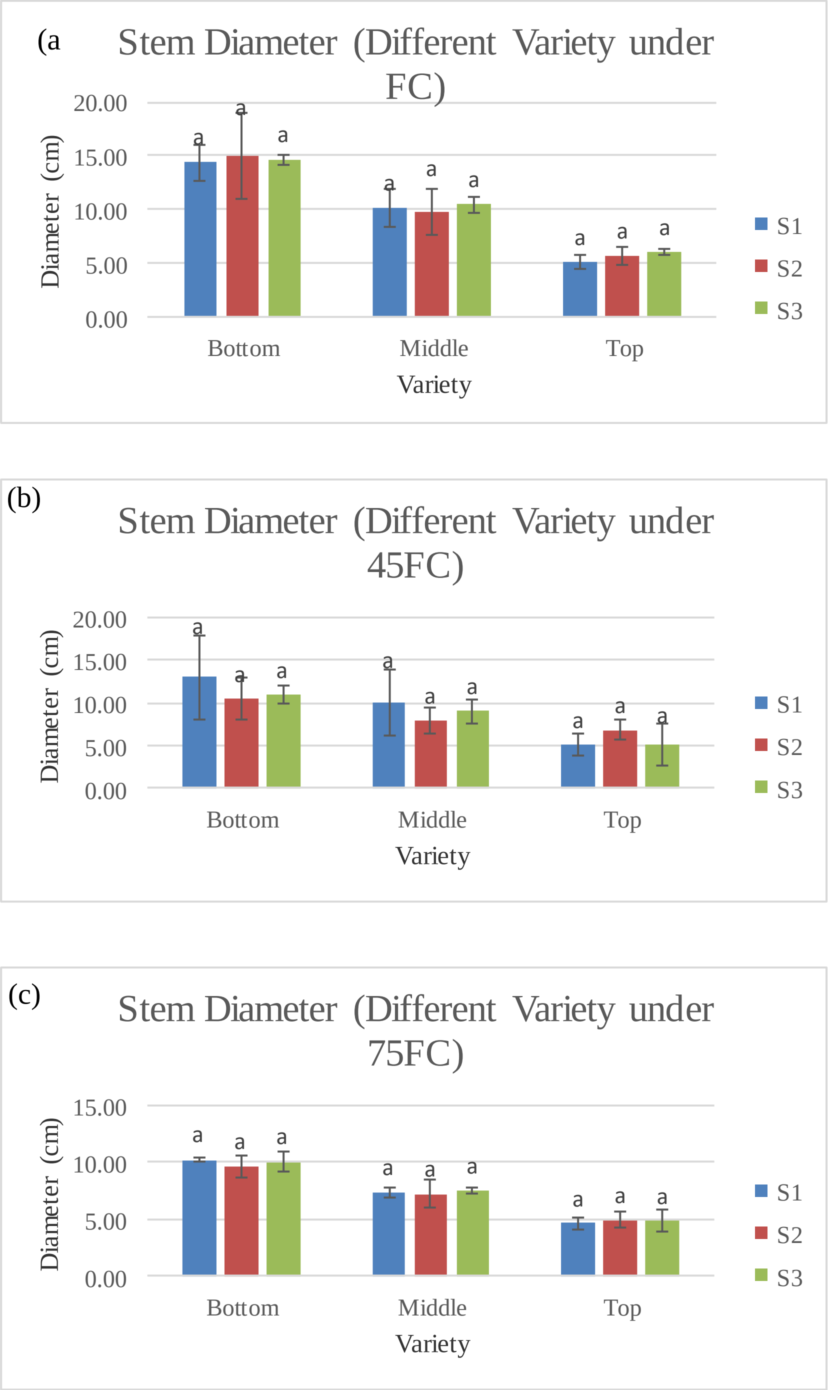


Figure 5: Average stem diameter for each individual variety population in different water treatments, (a) FC, (b) 45FC and (c) 75FC

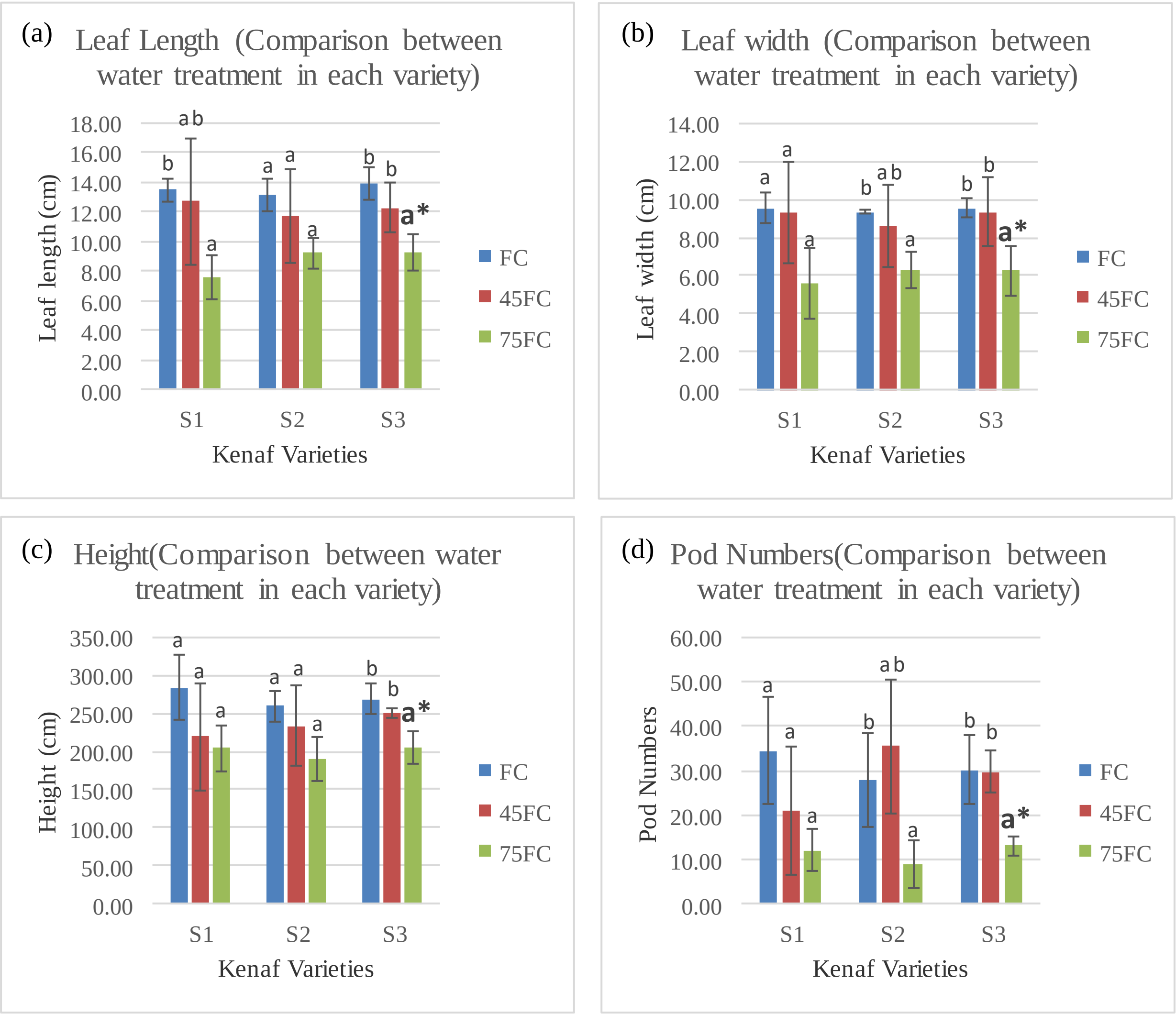


Figure 6: Average leaf length (a), leaf width (b), plant height (c) and pod numbers (d) of each individual variety population in different water treatments. **a*** indicates significant difference ($p < 0.05$) compared to other varieties

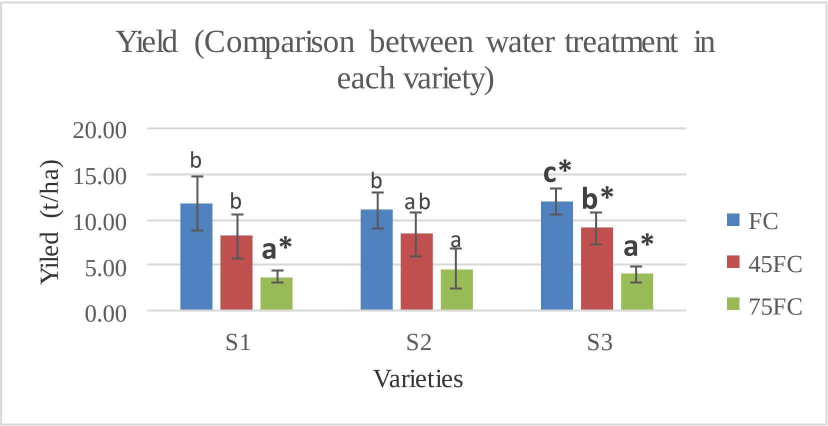


Figure 7: Average plant yield of separate variety under different water treatment. **a*, b*** and **c*** indicate significant differences ($p < 0.05$) compared to other varieties

## Nitrogen Use Efficiency

Figure 8 (a) depicts the graph to compare the average of samples NUE in different water treatments. The highest plant NUE was generated through 45FC water treatment with an average of 34.24%. 75FC was noted as the lowest NUE ($p < 0.05$) obtained as compared to FC and 45FC. Figure 8 (b) shows that the highest NUE was noted in S3 mutant populations under FC water treatment ($p < 0.05$) as compared to other varieties.

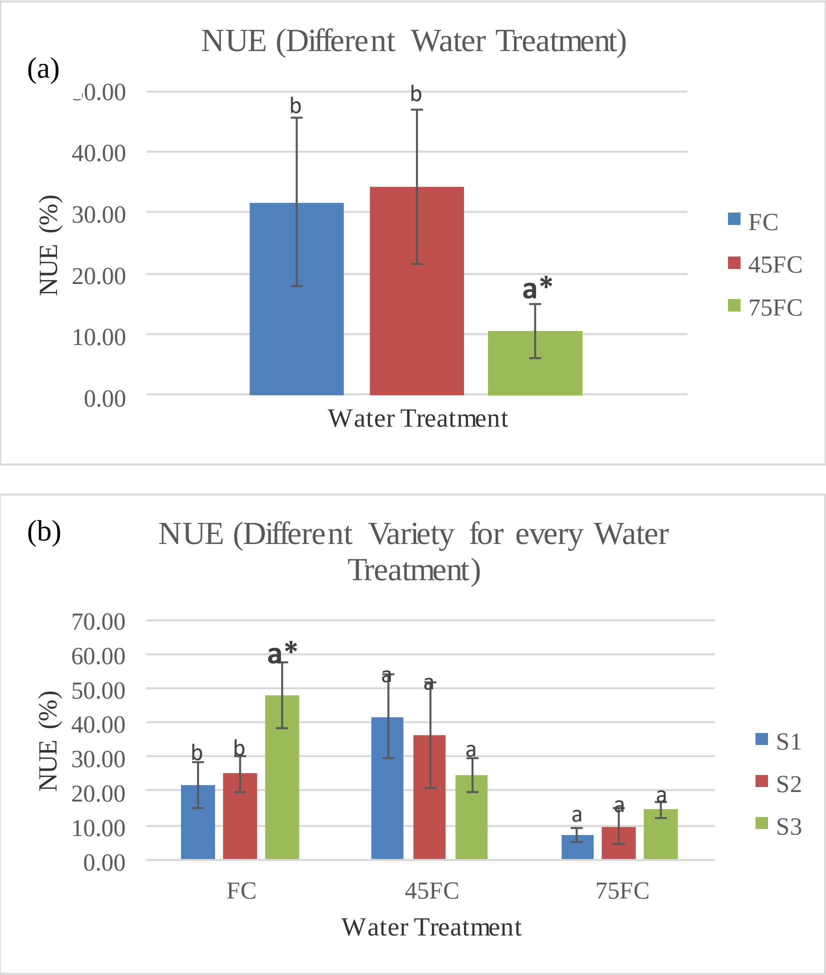


Figure 8: (a) Mean values of NUE in kenaf under different water treatments; (b) average plant NUE of all varieties under different water treatment. **a*** indicates significant difference ($p < 0.05$) compared to other water treatments (a) and varieties (b)

## NUE and $\Delta^{13}C$ Relationship

Figure 9 shows a relationship between NUE and $\Delta^{13}C$ of kenaf under different water treatments. From the result, a strong linear correlation relationship between NUE and $\Delta^{13}C$ of kenaf was noted ($r = 0.956$) in the study.

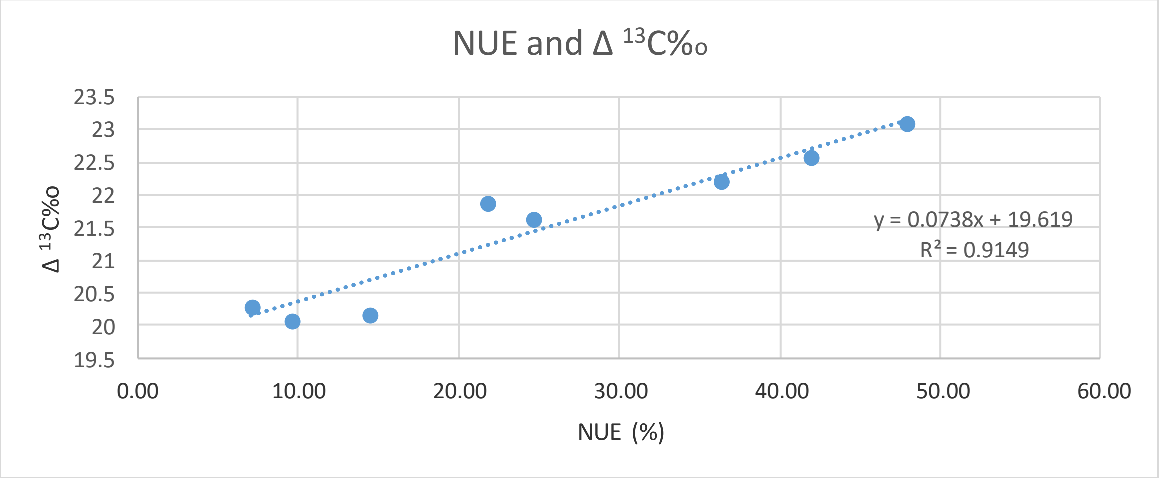
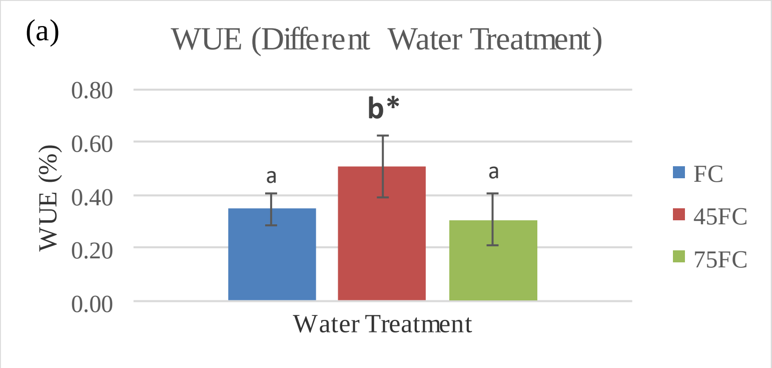


Figure 9: The relationship between NUE and $\Delta^{13}$C of kenaf under different water conditions

## Water Use Efficiency

Figure 10 (a) shows the graphs for average of WUE in different water conditions. The 45FC resulted in significantly higher ($p < 0.05$) WUE as compared to other treatment. Individually, S3 mutant population under 45FC has a higher ($p < 0.05$) WUE as compared to S3 population under others water condition (Figure 10 (b)).



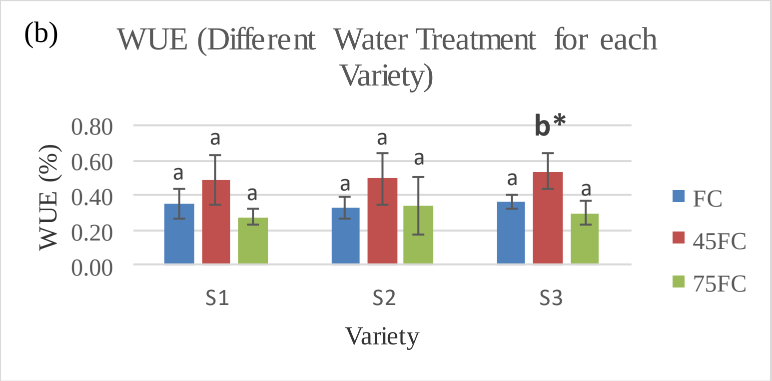


Figure 10: (a) Mean values of WUE in kenaf under different water treatments; (b) average plant WUE of separate variety under different water treatment. **b*** indicates significant difference ($p < 0.05$) compared to other water treatments (a) and varieties (b)

## Carbon Isotope Discrimination

As shown in figure 11 (a), 75FC significantly decreased ($p < 0.05$) $\Delta^{13}C$ value as compared to other water treatments. From the graph, the 75FC generated the lowest ($p < 0.05$) $\Delta^{13}C$ value for all varieties (Figure 11 (b)). Individually, S2 and S3 mutant population showed a significantly higher ($p < 0.05$) carbon isotope discrimination under FC treatment (Figure 11 (b)).

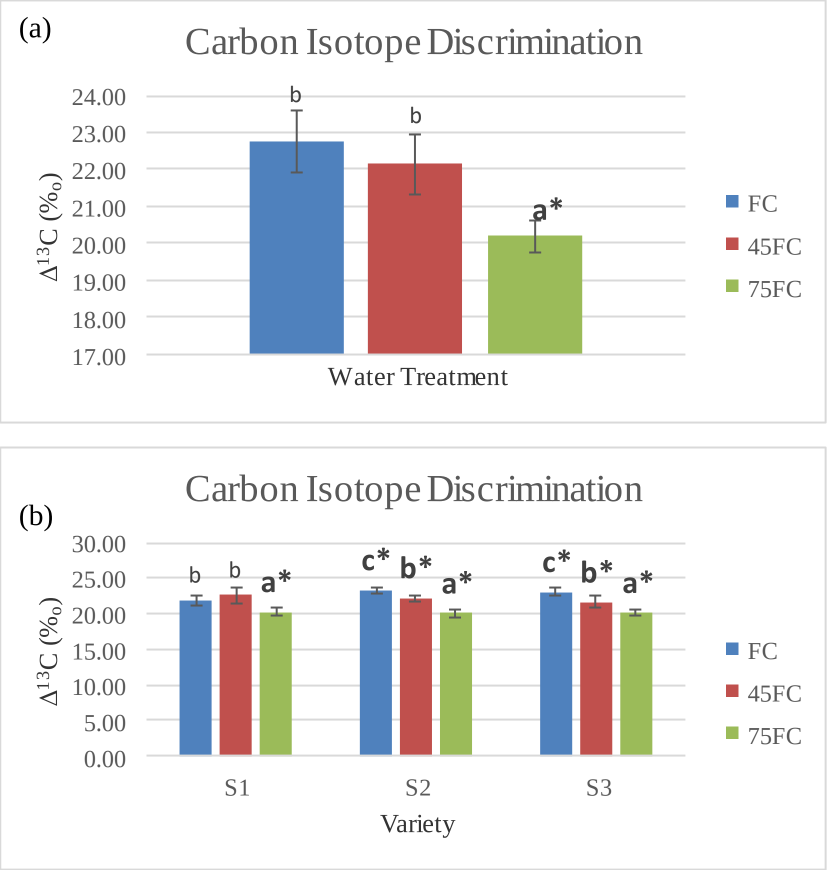


Figure 11: (a) Average $\Delta^{13}C$ values in different water treatment; (b) Average carbon isotope discrimination values of separate variety under different water treatment. **a***, **b*** and **c*** indicate significant difference ($p < 0.05$) compared to other water treatments (a) and varieties (b)

## $\Delta^{13}C$ and WUE Relationship

Figure 12 shows a relationship between $\Delta^{13}C$ and WUE of kenaf under FC, 45FC and 75FC water treatments. From the result, a weak linear correlation relationship between $\Delta^{13}C$ and WUE of kenaf was noted ($r = 0.460$) in the study.

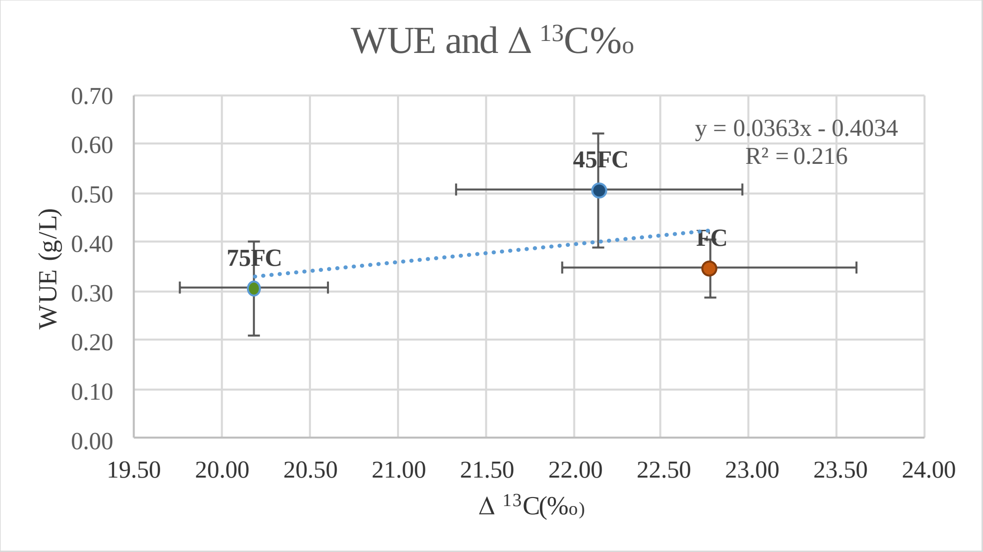


Figure 12: The relationship between $\Delta^{13}$C and WUE of kenaf under different water conditions

## DISCUSSION

Plant stem diameter, leaf development, plant height, pod numbers and plant yield are the appropriate agronomical characters to evaluate kenaf growth. The values of the agronomic parameters are very useful to determine the total biomass yield of the plant. From the study, the highest plant growth was obtained from FC water treatment followed by 45FC and 75FC accordingly. The 75FC was found to reduce the growth development of kenaf. It has been known that water stress is an important limiting factor at the initial phase of plant growth and establishment. Fibrous plant potential should be measured by the final value of plant height and basal diameter because these parameters are generally dependable yield components of bast fiber crops (Ogbonnaya et al., 1998).

Water stress greatly suppressed cell expansion and cell growth due to low turgor pressure (Jaleel et al., 2009). Therefore, the lowest plant height and stem diameter in the study were associated with a decline in the cell division in the shoot apical and lateral meristem respectively. The first process affected by changes in temperature or plant water status is leaf development (Saab and Sharp, 1989). The causes of reduction in leaf growth from this study could be associated with hydraulic or non-hydraulic. According to Sharp (2002), non-hydraulic process has an important role under water deficit involving abscisic acid since this hormone plays significant role in plant responses to water stress via a large number of processes. Among the factors limiting the leaf growth under water deficit by the hydraulic process are decrease in cell turgor which reduces the driving force of cell expansion and change in the gradient of water potential between the xylem and growing cells (Tardieu et al., 2010). Besides, change in aquaporins activity also affects the leaf elongation rate (Tardieu et al., 2010). Aquaporins are proteins that facilitate the water transport through membranes, thereby increasing the hydraulic conductivity of tissues when their channel is open and rapidly respond to surrounding conditions (Maurel et al., 2008). The production of low pod numbers in 75FC as compared to other treatments were probably due to a decrease in soil moisture level during the reproductive period. Water stress could delay cell division in the meristem and the transport of nutrients to different plant tissues (Nicholas et al., 1985). This includes younger buds that initially failed to mature, with unopened sepals that inhibited the extension of internal organs (Su et al., 2013). Consequently, the numbers of flower in a plant will be decreased. Thus the yield of kenaf in relation to its whole plant weight will be affected the reduction in vegetative development under severe water stress conditions.

It was observed from this experiment from that 75FC water stress treatment had significantly reduced the leaf growth, plant height, pod numbers and plant yield of S3 mutant line as compared to 45FC and FC treatments. The results were in accordance with Khalatbari (2015), who reported significant plant growth reduction on one of the tested kenaf varieties under severe water stress. Each plant develops different strategies for environmental stress adaptation (Nkaa et al., 2010). Therefore, among the varieties, S3 was able to generate the highest development on leaf, height, pod numbers and plant yield under the 75FC water condition as compared to S1 and S2 plants. In this study, S3 may be adopted more efficient mechanism of leaf rolling and stomatal closing to limit water loss as well as maintained the turgidity for cell enlargement in comparison with others. However, for plant stem diameter growth, there were no significant differences for the tested varieties under all stress treatments. These results were in accordance with those of Ohashi et al. (2009) who found the imposition of water stress does not affect the increment of the stem diameter due to the potential of the plant stem to conserve assimilates as a buffering stock in the presence of stress.

Among all three water treatments, 75FC was found to lower the nitrogen used in the plants significantly. Shortage of water supplies under water stress usually decreases the uptake of total nutrients and lowers tissue concentrations in plants (Saud et al., 2017). Such influences may also be associated with restricted energy accessibility for $NO_3^-$ / $NH_4^+$ under water deficit conditions (Farooq et al., 2009). Individually, S3 mutant population showed a significantly high NUE value under well watered conditions (FC) as compared to other varieties in the study. Most nutrients are taken up into the plant in forms of soluble inorganic fertilizers by the root system (Baligar et al., 2001; Fageria et al., 2002). Therefore S3 variety was believed to have a better and more efficient root system to absorb nutrient and water into the plant. Furthermore, we found that NUE has a strong direct correlation with $\Delta^{13}C$. This was also verified that in plants, nitrogen availability drives the proper photosynthetic functional activity of the leaf (Brennan, 1992).

From the study, the highest value of WUE was obtained through 45FC treatment which represents moderate stress condition. In fact, this treatment did not adversely affect the growth of kenaf varieties at the end of the experiment. Medrano et al. (2015) found that the WUE of grape vines were higher under moderate water stress condition in their study on scaling up from single-leaf to whole-plant WUE by comparison of daily integrals of photosynthetic water use efficiency. Daily leaf WUE proved to be highly determined by daily intercepted light at each leaf position (Medrano et al., 2015). Among the varieties, the S3 kenaf mutant population significantly possessed highest WUE under 45FC treatment as compared to other tested varieties. Underwater stress, osmotic adjustment is a major cellular water stress-responsive trait that contributes to cellular dehydration avoidance then used water efficiently (Blum, 2005).

Plants preferably take up lighter $^{12}CO_2$ isotopologue and discriminate against the heavier $^{13}CO_2$ isotopologue. It is called photosynthetic carbon isotope discrimination ($\Delta^{13}C$), results in general $^{13}C$ depletion of the terrestrial biosphere compared with atmospheric $CO_2$ (Brugnoli and Farquhar, 2000), and is used for applications at different spatial and temporal scales (Gentsch et al., 2014). The principle for this is that carbon fixed by the photosynthetic $CO_2$ assimilating enzyme RUBP carboxylase (Rubisco) in $C_3$ plants and PEP (Phosphoenolpyruvate) carboxylase in $C_4$ and CAM plants show different isotope fractionation $\delta^{13}C$ values (Pate, 2001). From the study, lowest $\Delta^{13}C$ is significantly derived from 75FC water treatment. All the varieties treated with 75FC showed significantly low $\Delta^{13}C$ results. Since carbon isotope discrimination is strongly connected to the photosynthetic values of plants, under severe water stress condition (75FC), the plants growth and yield would be lower as compared to well-watered kenaf.

The results of the study show a weak correlation between $\Delta^{13}C$ and WUE ($r$ = 0.460). This relationship confirms to the findings by Elazab et al. (2012) where $\Delta^{13}C$ has been proven as an indirect indicator for WUE. Intrinsic WUE can be evaluated at leaf level as the ratio of $CO_2$ exchange rate of transpiration (Morgan et al., 1993) or the ratio of biomass produced to transpiration (Akhter et al., 2008). As a $C_3$ plant, the physiological basis for $\Delta^{13}C$ variation in kenaf is related to the variation in the internal $CO_2$ concentration (*Ci*) to ambient $CO_2$ concentration (*Ca*) ratio. High $\Delta^{13}C$ values resulting from high *Ci*/*Ca* reflect higher $CO_2$ assimilation rate to transpiration ratio (Farquhar et al., 1989) then slightly higher the WUE in the tested kenaf plants. It means, photosynthesis in kenaf will relatively increase with the efficient use of water by the plant.

## CONCLUSIONS

Based on the findings from this study, the FC water treatment was suggested as the best application to grow kenaf plant in order to obtain higher biomass (longer plant/stem, high pod numbers, bigger leaves and plant yield). The application of 75FC, particularly for the tested kenaf varieties not recommended because it can result in lower biomass as well as lower yield. Among the varieties, S3 mutant line was used the nitrogen efficiently under FC condition. The 45FC treatment was suggested as the best application to achieve higher WUE in the kenaf varieties. The S3 mutant line was recommended to grow under 45FC especially in areas with water shortage problem to reduce costs and avoid wastage of water since it does not adversely affect the biomass, yield values of the plant. We recommended that $\Delta^{13}C$ to be used as a tool to determine the plant growth response because it is associated with the efficiency of photosynthesis processes. WUE was clearly linked with $\Delta^{13}C$ as it has been indicated in this study and previous reports.

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