

Multivariate statistical analyses for studying kenaf germination, growth, and fiber production under salinity constraint

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Abstract: It is due to the lack of renewable fiber sources for industrial purposes that the need for finding available and alternative material has become imperative. Kenaf (*Hibiscus cannabinus* L.), a fast-growing industrial crop, would be a potential solution for valorizing salinized lands and/or soils irrigated with saline water. With the aim of selecting and valorizing salt-tolerant species with high fiber yields and industrial values, we launched the assessment of the performance of kenaf culture (var. Guangdong 743-2) under salinity constraints. Germination and vegetative stages were considered in the present study to better evaluate the whole life cycle of the species. Several NaCl concentrations (0, 3, 6, 9, 12, and 15 g L⁻¹) were applied to seeds cultivated in petri dishes and to 4-month-old plants growing in hydroponics. Germination rates (germination capacity and coefficient of velocity), growth characteristics (biomass production and relative growth rate), physiological parameters (ionic content and water status), and fiber yields (neutral detergent fiber, acid detergent fiber, and acid detergent lignin) were evaluated to better understand the salt stress and toxic effects on germination, growth, and fiber yield. Multivariate analyses were used to identify the major characteristics pertaining to salinity tolerance. The obtained results have shown that kenaf variety Guangdong 743-2 is able to germinate and grow under high salinity levels (up to 15 g L⁻¹ NaCl), deploying several mechanisms of adaptation. Kenaf could withstand salt stress by germinating smoothly, preserving root and stem biomass, maintaining relative growth rate, stabilizing root water content, producing new leaves while sacrificing older ones, and behaving as a halophyte species through the inclusion of toxic ions within the aerial part, most probably compartmentalized inside vacuoles to ultimately keep its fiber yields unchanged.

Key words: Cellulose, germination, lignin, salt stress, principal component analysis, multiple linear regression

1. Introduction

Despite the recent growth in plantations of fast-growing trees, the supply of fiber raw materials remains a major problem for the pulp industries in many countries of the world. Worldwide, cellulose production from wood represented 10% of the total pulp produced during 2000, and foresters estimated that during 2010 the rate reached 15%. Moreover, exploitation rates are expected to increase by 30% by 2020 as compared to 2010 (COM, 2013). Thus, pressure on forest lands is increasing to meet the industrial needs (Villar et al., 2009). Several countries, including Tunisia, are finding their paper industries seriously

threatened because of the lack of raw material and the decline of forest resources.

The use of substitute fibers is starting to be topical for manufacturers of paper in seeking possible sources of economic fiber. Indeed, although the production of printing paper by the National Society of Cellulose and Alfa Paper in Kasserine (west-central Tunisia) was up 21% to 27,000 t, local production of pulp, paper, and cardboard could not satisfy all domestic needs. Imports of these products, at 179,500 t for a value of 185.6 million Tunisian dinars, grew by 79% in 2001 (CEPI, 2003). Hence, the

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interest in nonwood lignocellulosic fiber resources has increased to assist the efforts of forest preservation.

In Tunisia, *Stipa tenacissima* (esparto) is currently used in the pulp and paper industry. Due to regeneration problems related to the severe climate, characterized by a long period of drought as well as insufficient and erratic rains in west-central Tunisia, esparto pulp production decreased (Belkhir et al., 2013). Kenaf could produce up to 30 t ha⁻¹ of dry stem material and yields approximately three to five times as much fiber as southern pine (Monti and Alexopoulou, 2013). Therefore, the introduction of kenaf in Tunisia could represent a renewable and promising source of natural cellulosic fiber and material for the paper industry. Kenaf (*Hibiscus cannabinus* L.) is a warm seasonal annual crop belonging to the family Malvaceae that grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall (Coetzee et al., 2008).

However, to the best of our knowledge, little or nothing is known about its seed germination and ecophysiology under saline conditions and environments. Indeed, soils affected by salinity in Tunisia cover about 1.5 × 10⁶ ha, representing 10% of the area of the country (Hachicha, 2007). Salinity is a major factor limiting plant productivity, especially in arid and semiarid lands where salt stress induces physiological and biochemical changes in plants (Gupta and Huang, 2014). The expansion of irrigated agriculture and intensive use of water resources, combined with high evaporation in arid and semiarid areas, inevitably leads to the salinization of soils and water sources, with an estimation that 50% of the arable land would be salinized by the year 2050 (Jamil et al., 2011). The alternative approach is to use plants that display some salt tolerance and adaptation behavior, whose yields remain satisfactory versus high levels of salt.

While the traditional use of kenaf focuses on kenaf's fiber production, new applications have recently been developed such as pulping, papermaking, and board making. It has also been identified as an excellent source of cellulosic fiber for the manufacturing of a large range of paper products (Monti and Alexopoulou, 2013), which would satisfy the new and shifting industrial needs and requirements.

In this context, the objective of the present work is threefold. It first aims to assess the behavior of kenaf under salt stress, second to highlight its ability to maintain its fiber yield even under salinity constraints, and finally to determine its suitability for our pedoclimatic conditions. Since germination is one of the most critical phases in plant culture establishment, we planned first to assess the degree of salt tolerance at the germination stage and then to study the salinity effect at the vegetative stage under hydroponic conditions with particular attention on fiber

yield. To this end, we aimed to evaluate salt tolerance at the germination stage and vegetative stage along with the fiber yield of kenaf using multivariate analyses.

2. Materials and methods

2.1. Plant material

In this study, we used the Chinese kenaf variety Guangdong 743-2, renowned for its high fiber yields (30 t ha⁻¹).

2.2. Germination procedure

Seeds were surface-sterilized first by a fungicide treatment (1 g L⁻¹ Benlate) for 30 min, immersed in 6% calcium hypochlorite solution for 5 min, and then rinsed in 70% ethanol for 5 min and thoroughly washed with sterilized distilled water. Seeds were plated onto plastic petri dishes (9 cm in diameter) containing sterile perlite and placed in a growth chamber where the temperature and humidity were 25 °C and 80%, respectively, with a photoperiod of 16 h day⁻¹. Lighting was provided by OSRAM L36W/77 type lamps (FLUORA, white fluorescent tubes) providing an intensity of 1500 μmol h⁻¹ photon⁻¹. Each petri dish contained 20 seeds with five replicates per treatment. The experimental design was arranged in a completely randomized block comprising 6 concentrations of NaCl salt. Germinated seeds were counted daily during 1 week using radicle protrusion (2 mm) as a criterion for germination. The parameters of the germination capacity (GC) and germination kinetics, i.e. the mean time of germination (MTG) and the coefficient of velocity (CV) of Kotowski (1962), were calculated as follows:

· Germination capacity:

$$GC (\%) = \frac{n}{N} \times 100$$

Here, n is the total number of germinated seeds and N is the total number of tested seeds.

· Mean time of germination (MTG): time (t_{1/2}) required to achieve 50% of germination, calculated by interpolation from the cumulative germination curve.

· Coefficient of velocity: $CV = \sum (n_i \times D_i) / \sum n_i$

Here, n_i is the number of seeds newly germinating on day i and D_i is the number of days from sowing.

2.3. Vegetative stage

Salt stress treatment was performed on adult 4-month-old plants obtained from germination. Plants were of homogeneous appearance with simple and lobed leaves.

2.4. Salt stress treatment

The salt stress treatment was conducted under hydroponic conditions using modified Knop nutrient solution supplemented with Sequestrene solution (1 mL L⁻¹). Salt treatment consisted of 6 NaCl concentrations applied to the nutrient solution (0, 3, 6, 9, 12, and 15 g L⁻¹ NaCl) with 10 replicates per treatment. The duration of treatment was 35 days to reach 5-month-old plants, which is the maturity stage.

2.5. Parameter calculations and expression of results

2.5.1. Morphological parameters

Observations of plant morphology and the monitoring of toxicity symptoms were recorded in a regular way during the period of treatment.

2.5.2. Growth parameters

Biomass production measurements were performed at two culture points: the beginning (initial harvest) and the end (final harvest) of treatment. The fresh weight of all organs (leaves, stem, and roots) was measured after harvest. Dry weight was obtained after drying at 60 °C for 48 h. The measures of fresh (FW) and dry weight (DW) enabled us to follow the evolution of the total biomass and growth in stressed plants compared to the control.

2.5.3. Relative growth rate (RGR)

The amount of dry matter produced during a whole treatment depends on the initial plant size and its growth within this period. The RGR is the average dry matter production per time unit and per biomass unit. It is expressed in day⁻¹ and calculated using the following formula (Hunt, 1990):

$$RGR = (\ln DM_f - \ln DM_i) / (t_f - t_i)$$

DM_f: Dry matter yield (g) (whole plant or organ) at the final harvest.

DM_i: Dry matter yield (g) (whole plant or organ) at the initial harvest.

$$t_f - t_i = \text{Treatment time (days) for final and initial.}$$

The RGR has the advantage of providing a better estimate of the effects of salt on the growth activity during treatment.

2.5.4. Sensitivity index (SI)

The SI is calculated according to the following formula (Sleimi, 2002):

$$SI = 100 \times ((\Delta DM_{NaCl} - \Delta DM_{control}) / \Delta DM_{control})$$

ΔDM_{NaCl}: Variation of dry matter production under NaCl treatment.

ΔDM_{control}: Variation of dry matter production under control treatment.

2.5.5. Water content (WC)

The WC is calculated as the ratio of the amount of water contained in the sample to the dry weight of the sample:

$$WC = \frac{(FW - DW)}{DW} \text{ (mL/g DW)}$$

FW (mg) = Fresh weight material.

DW (mg) = Dry weight material.

2.5.6. Mineral nutrition status

In order to follow the pattern of ion accumulation in the whole plant, we determined the ionic content of Na⁺, Cl⁻, and K⁺ within leaves, stems, and roots. Thus, the samples from different organs were ground to a fine powder with a mortar and pestle using liquid nitrogen. After drying for 48 h at 70 °C, 25 mg of dried powder was digested

in 30 mL of nitric acid (0.5%), then filtered using filter paper and diluted with distilled water for mineral element content analysis. K⁺ and Na⁺ contents were determined with atomic absorption spectroscopy, while Cl⁻ content was determined from the same acid extracts supplemented with acetic buffer (10% acetic acid and 0.1 N nitric acid) and gelatin, using a digital chloridometer (Haake Buchler type) according to the principle of colorimetric titration (Abdelly, 1997).

2.5.7. K⁺/Na⁺ selectivity

The K⁺/Na⁺ selectivity is calculated by the following formula:

$$S_{K^+/Na^+} = S1 / S2$$

$$S1 = \Delta QK / (\Delta QK + \Delta QNa).$$

$$S2 = [K^+] / ([K^+] + [Na^+]).$$

ΔQ: Amount of element accumulated within the organ during the treatment period (mEq).

[...]: Element concentration within the organ during the treatment period (mEq mL⁻¹).

2.6. Fiber content determination

The fiber content is measured according to the method of Van Soest et al. (1991) based on sequential analysis, for which the following fibers are determined:

- ♦ Neutral detergent fiber (NDF), representing the total plant cell wall matrix, which includes cellulose, hemicellulose, and lignin as the major components.
- ♦ Acid detergent fiber (ADF) or lignocellulose, formed by lignin and cellulose.
- ♦ Acid detergent lignin (ADL) or crude lignin.

The principle of this method consists of adding to the fiber sample a lauryl sulfate (NDS), acid detergent (ADS), or 72% sulfuric acid-based solution to solubilize the cellular content and keep only the wall residues of NDF, ADF, and ADL. ANKOM Technology equipment was used to perform the sequential analysis of the NDF, ADF, and ADL fractions. Fibers (NDF, ADF, and ADL) are expressed in percentage of dry weight (after drying the samples at 105 °C for 24 h). The amounts of hemicellulose, cellulose, and lignin are obtained according to the following formulas:

$$♦ \text{NDF} = \text{Hemicellulose} + \text{cellulose} + \text{lignin}$$

$$♦ \text{ADF} = \text{Cellulose} + \text{lignin}$$

$$♦ \text{ADL} = \text{Lignin}$$

$$♦ \text{Hemicellulose} = \text{NDF} - \text{ADF}$$

$$♦ \text{Cellulose} = \text{ADF} - \text{ADL}$$

2.7. Statistical analysis

The data of the different parameter values were subjected to one-way ANOVA with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The differences between the means were tested with the Student–Newman–Keuls test and values of P ≤ 0.05 were considered significantly different (Sokal and Rohlf, 1995).

Principal component analysis (PCA) of phenotypic data was performed with the R language. An absolute

value of 0.50 was used in the loading matrices to select the characteristics in a particular principal component (PC). Correlations between variables were calculated with Spearman correlation coefficients.

Multiple linear regression (MLR) techniques based on least-square procedures are usually used for estimating the variable effects involved in a model. In this study, MLR was carried out on the training data set, using GC, RGR, SI, stem (K^+/Na^+), and leaves (K^+/Na^+) as the response variables and other parameters as predictor variables. The success of MLR can be measured by evaluating the magnitude of the adjusted R^2 , the residual standard error (RSE) for the regression, and the results of the Student t-test for the individual predictor variables.

3. Results and discussion

3.1. Effect of salt stress on germination

3.1.1. Germination capacity

The results for the germination behavior of kenaf seeds in terms of GC under the effect of different NaCl concentrations are shown in Table 1. Interestingly, kenaf germination rate was slightly affected by salinity and seeds were able to germinate at up to 12 and 15 g L⁻¹ NaCl at the rates of 74% and 40%, respectively. This indicates that kenaf seeds have a high GC even under high salt stress. According to the statistical analysis, there was no significant difference between the control and the plants subjected to 3 and 6 g L⁻¹ NaCl treatments, whose germination rate reached 88%. However, although salinity slowly reduced the rate of kenaf germination, the latter dropped to 40% when treatment reached 15 g L⁻¹ NaCl. The results of the analysis of variance confirmed that the salinity levels influenced the percentage of germination and the mean germination time. Mean germination rate comparison did not reveal any significant difference between 6, 9, and 12 g L⁻¹ NaCl treatments. Moreover, only the 15 g L⁻¹ NaCl level of salinity was considered as the worst treatment and significantly different from the others.

Table 1 . Germination characteristics of kenaf seeds under different NaCl concentrations.

NaCl (g/L)	0	3	6	9	12	15
GC	94A	88A	88A	83B	74B	40C
MTG	1.5	1.5	1.5	1.5	1.5	-
CV	50a	50a	50a	49a	43b	29c

GC: Germination capacity (%), MTG: mean time of germination (days), CV: coefficient of velocity. Values with the same letter in the same line are not significantly different ($P \leq 0.05$).

3.1.2. Kinetics of germination

The calculated average values of 5 replicates per treatment for different salinity levels (0, 3, 6, 9, 12, and 15 g L⁻¹ NaCl), allowed us to draw the germination percentage curves as a function of time (Figure 1) and to derive the mean germination time for each salinity level. Figure 1 reveals the general pattern of a sigmoidal curve with a short lag phase (1 day), an exponential phase (1–2 days), and a stationary phase where the germination rate stabilizes. The general tendency of kenaf seed germination, even under salinity constraint, follows a swift kinetics. Just after 2 days of sowing, the stationary phase of germination was reached for levels below or equal to 9 g L⁻¹ NaCl, whereas for higher levels of salinity (12 and 15 g L⁻¹ NaCl) it was obtained after 4 days. Moreover, MTG was around 1.5 days for salinity below or equal to 12 g L⁻¹ NaCl, and CV was maintained elevated even under high NaCl concentrations, showing the velocity of germination (Table 1).

The interactions between germination parameters under stress conditions were studied by multivariate analysis, PCA. Regarding the PCA performed for NaCl treatments, by considering 3 parameters, the first two components (F1 and F2) explained 97% of the total variation. The first component (axis 1) explained 91% of the variation, followed by 6% for the second component (axis 2) (Figure 2).

Likewise, the Spearman correlation matrix clearly showed that NaCl was correlated positively with MTG ($R^2 = 0.800$) and negatively with GC and CV ($R^2 = -0.800$ and -0.600 , respectively).

These results were confirmed by MLR analysis. The results from MLR were obtained using GC as the independent variable and NaCl, CV, and MTG as dependent variables. This was done to determine the best linear combination of the constructs for predicting attitude. We show that 99.7% of the variance in the model can be

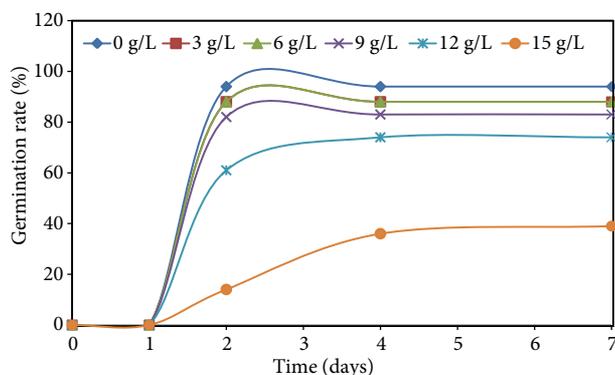


Figure 1. Kinetics of kenaf seed germination under different NaCl concentrations.

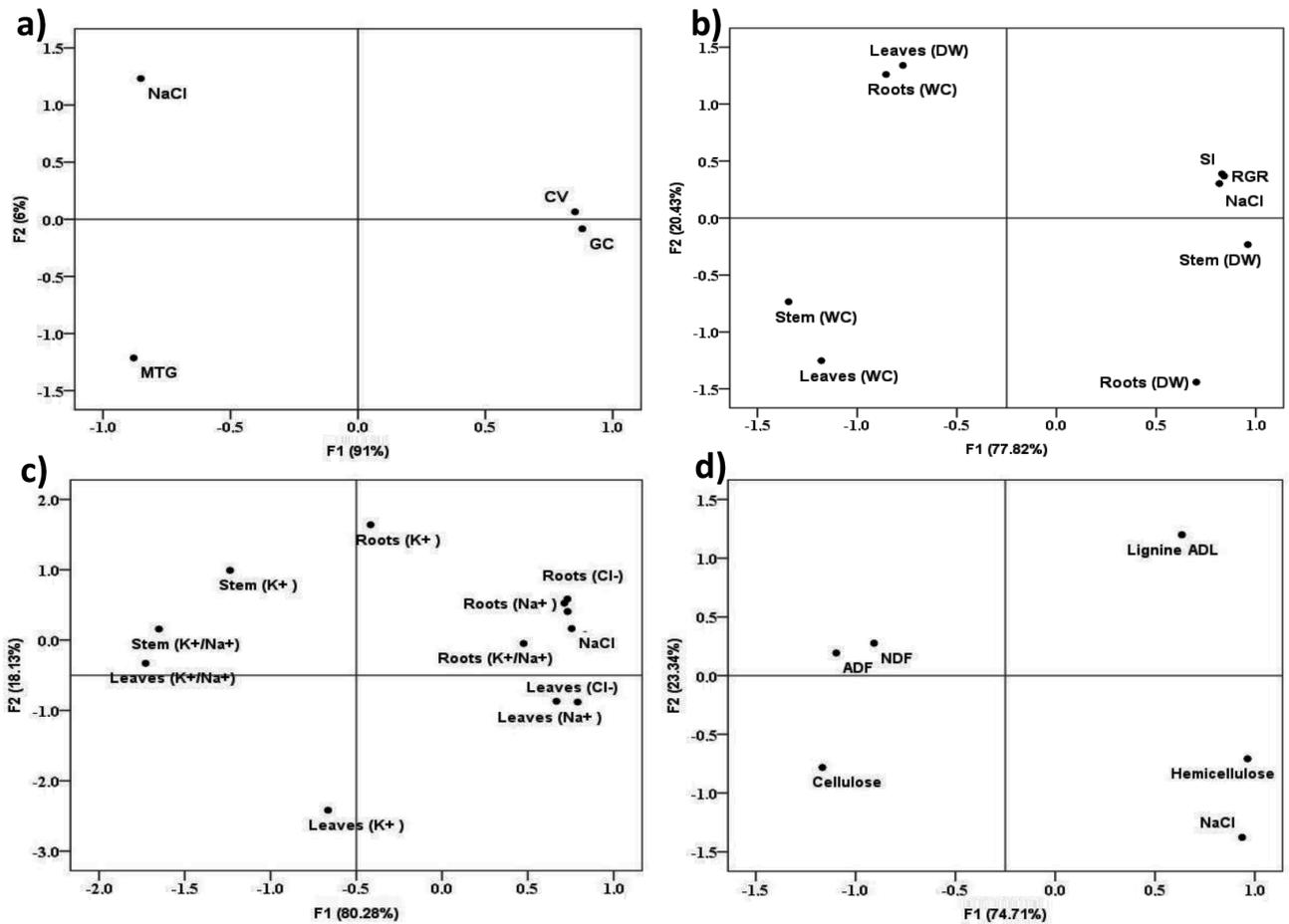


Figure 2. Principal component analysis of the germination parameters (a), plant growth (b), mineral nutrition (c), and fiber yields (d) of kenaf (*Hibiscus cannabinus* L.) under saline constraints.

predicted using the independent variables. Additionally, MLR analysis shows the standardized beta coefficients that present the contributions of each variable to the model. The t - and P -values show the impact of the independent variables on the dependent variable. All independent variables are insignificant except for CV, where $P = 0.013$. The large t -value ($t = 8.53$) and the corresponding significant P -value ($P < 0.05$) support the result for CV, which has the highest beta coefficient ($\beta = 1.647$). On the other hand, there were no significant relationships between GC and NaCl or between GC and MTG, with negative t -values ($t = -1.33$, $t = -2.55$, respectively). The value of the determination coefficient ($R^2 = 0.997$) indicates that 99.7% of the variability in the response could be explained by the model. This result is confirmed by the ANOVA analysis for the estimated models of GC. As the P -value is 0.005, the model is significant. Thus, the combination of the variables significantly predicts the dependent variable ($F = 207.24$; $P < 0.05$).

3.2. Effect of salt stress on plant growth

3.2.1. Morphology of plants

The adverse effects of salts occur at the whole plant level, being more visible within older leaves. The salt effect on kenaf plants appeared starting from the 20th day of culture for the higher salinity levels (12 and 15 g L⁻¹ NaCl), although the first symptoms could be observed at 9 g L⁻¹ NaCl on some plants. The symptoms of toxicity become visible in both aerial parts with necrotic leaves and stems and within roots turning brown. For 3, 6, and 9 g L⁻¹ NaCl treatments, the roots were still white but small necrotic spots in older leaves spread from the margins inwards. However, at higher doses, some roots turned brown and necrosis occurred at the tip and margin of leaves that eventually withered, a sign of toxicity excess through salt buildup. Older chlorotic leaves usually become necrotic in late stages and they frequently fall off the plant while the newest younger leaves are regenerated.

3.2.2. Biomass production

In order to assess the effect of salt stress on vegetative yield, the fresh and dry weights of kenaf plants were examined under both control and stressed conditions for the different organs: roots, stems, and leaves (Table 2). Usually, as the salt concentration increases, the yield is expected to decrease. However, remarkably, kenaf plants were not significantly affected by salinity. Up to 15 g L⁻¹ NaCl, salt treatments did not considerably inhibit the plant growth, causing no substantial decrease in both fresh and dry weights, particularly for roots and stems, while leaves showed a reduction in fresh and dry weight starting from 3 and 6 g L⁻¹ NaCl, respectively. Despite showing an increase in stem dry weight under all salt treated conditions, obviously, these differences are not significant. Such results have been obtained by several authors who reported an increase in fresh and dry weight shoot material under NaCl treatment (Pessarakli et al., 2001; Taffouo et al., 2009; Memon et al., 2010; Amira, 2011). Most importantly in our findings is that stem biomass, which is positively correlated with fiber production, is maintained (even increased) under high levels of NaCl concentration.

3.2.3. Relative growth rate and sensitivity index

To better understand the effect of salt stress on seedling growth, the RGR of the whole plant was determined. The calculations of RGR throughout the experimental period revealed that kenaf plants are able to maintain growth activity for all salinity levels and even increase it for 12 and 15 g L⁻¹ NaCl (Table 2). The values of SI are all positive (Table 2), indicating a growth stimulation compared to control plants and showing that kenaf behaves as a tolerant species.

3.2.4. Tissue hydration

WC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water

deficit due to osmotic stress. Regardless of the culture conditions, the WC of the roots is slightly higher than that of the stems and leaves, probably due to the hydroponic culture system. In the presence of salt, the root WC is more or less unchanged (Table 2). Indeed, although it increases to the level of 8.25 mL g⁻¹ DW for 3 g L⁻¹ NaCl, it remains moderately stable compared to the control for the other salinity levels (9, 12, and 15 g L⁻¹). In both stems and leaves, WC dropped significantly starting from 3 g L⁻¹ NaCl and stabilized, with leaves being the most affected by dehydration. Kenaf reacts to salt stress by protecting and keeping unchanged its root WC, even lowering its aerial WC.

The study of the effect of salt stress on plant growth was completed by multivariate analysis, PCA. Regarding the PCA performed for NaCl treatments, by considering 8 parameters, the first two components (F1 and F2) explained 98.25% of the total variation. The first component (axis 1) explained 77.82% of the variation, followed by 20.43% for the second component (axis 2) (Figure 2).

Similarly, the Spearman correlation matrix clearly showed that NaCl was correlated positively with RGR ($R^2 = 0.883$), stem DW ($R^2 = 0.833$), and SI ($R^2 = 0.867$) and negatively with stem WC and leaf WC ($R^2 = -0.817$ and $R^2 = -0.983$, respectively).

These results were confirmed by MLR analysis. The results from the MLR were obtained using NaCl, root DW, stem DW, and leaf DW as dependent variables and RGR (model 1) or SI (model 2) as the independent variable.

This was done to determine the best linear combination of the constructs for predicting attitude. The results show that 99.9% of the variance in either model 1 or model 2 can be predicted using the independent variables. The obtained results present the standardized beta coefficients that present the contributions of each variable to the two models.

Table 2. Influence of different NaCl concentrations on growth parameters of kenaf plants.

NaCl (g/L)		0	3	6	9	12	15
DW (g)	Roots	0.47a	0.48a	0.60a	0.61a	0.56a	0.53a
	Stem	2.66a	2.74a	3.08a	2.90a	3.19a	3.06a
	Leaves	0.55a	0.52a	0.24a	0.33a	0.50a	0.55a
RGR (10 ⁻³ day ⁻¹)	Whole plant	3.83	4.28	5.63	5.08	7.89	7.20
SI		-	1.58	6.49	4.46	15.25	12.52
WC (mL/g DW)	Roots	6.78b	8.25a	5.85b	6.28b	6.71b	6.98b
	Stem	6.21a	4.56b	4.24b	4.86b	3.98b	4.34b
	Leaves	6.00a	1.65b	2.35b	3.21b	1.41b	0.70b

DW: Dry weight, RGR: relative growth rate, SI: sensitivity index, WC: water content. Values with the same letter in the same line are not significantly different ($P \leq 0.05$).

The *t*- and *P*-values show the impact of the independent variables on the dependent variable. For the first model, all independent variables are insignificant, except for those of stem DW and leaf DW, where *P* = 0.007 and 0.024, respectively. Large *t*-values (*t* = 86.42, *t* = 26.95) and the corresponding significant *P*-value (*P* < 0.05) support the results for insignificance, except for stem DW and leaf DW, which had the two highest beta coefficients ($\beta = 6.83906$, $\beta = 6.3406$). The value of the determination coefficient ($R^2 = 0.999$) indicates that 99.9% of the variability in the response could be explained by the first model.

This result is confirmed by the ANOVA analysis for the estimated models of RGR. As the *P*-value is 0.007, the first model is significant. Thus, the combination of the variables significantly predicts the dependent variable (*F* = 10159.67; *P* < 0.05). Similar results were shown for model 2.

3.3. Effect of salt stress on mineral nutrition

3.3.1. Sodium content

Sodium concentrations increased in all analyzed organs as a function of salinity (Figure 3). Sodium was mostly accumulated in leaves. Beginning from 3 g L⁻¹ NaCl treatment, there was an uptake of sodium, mainly carried and stored in the aerial parts of the plant, especially in leaves. Nevertheless, within roots, sodium content increased significantly starting from 6 g L⁻¹ NaCl. Leaf sodium content doubled (4 mEq g⁻¹ DW) and tripled (5.7 mEq g⁻¹ DW) at 3 g L⁻¹ and 15 g L⁻¹ NaCl, respectively, in comparison with the control treatment (1.9 mEq g⁻¹ DW), highlighting the ‘includer’ behavior of kenaf under salinity constraints. Similarly, stems were able to accumulate a high level of sodium (4 mEq g⁻¹ DW) at 9, 12, and 15 g L⁻¹ NaCl. Even when increasing, the level of Na⁺ content within roots did not reach 2.5 mEq g⁻¹ DW under the most severe NaCl treatment (15 g L⁻¹), indicating that Na⁺ is mainly and highly overloaded in the aerial parts of the

plant. Sodium accumulation in the aerial parts of the plant, particularly in leaves, is performed at the cellular level by its compartmentalization inside the vacuole by a sodium/proton antiporter (Bassil and Blumwald, 2014; Hanana et al., 2007; Reguera et al., 2014).

3.3.2. Chloride content

The mineral analysis of chloride ions showed results similar to those recorded for sodium (Figure 3). Like sodium, the chloride content of different organs increased with the increase in salinity. The leaves accumulated the highest amounts of chloride. From the lowest concentration of NaCl (3 g L⁻¹), the level of chloride content was found to reach twice that of the control, with 4 mEq g⁻¹ DW, and it stabilized there up to 15 g L⁻¹ NaCl. Up to 6 g L⁻¹ NaCl, the chloride content of stems remained unchanged, but starting from 9 g L⁻¹ NaCl, the stems displayed similar chloride accumulation to leaves but with lower levels. Regarding roots, the levels of chloride contents increased slowly, even tripling from the control treatment (0.5 mEq g⁻¹ DW) to 15 g L⁻¹ NaCl (1.5 mEq g⁻¹ DW).

3.3.3. Potassium content

Under control conditions, the distribution of K⁺ (Figure 3) in aerial parts, which was around 1 mEq g⁻¹ DW for both leaves and stems, was higher than in the roots with 0.5 mEq g⁻¹ DW. At 3 and 6 g L⁻¹ NaCl, the foliar K⁺ contents increased a little bit and then decreased to the control value and stabilized for higher NaCl concentrations. The accumulation of potassium within kenaf leaves plays a crucial role in osmotic adjustment and helps in maintaining the turgor within the cell in response to osmotic stress due to NaCl application (Gupta and Huang, 2014). Potassium content within stems slowly decreased to 0.8 mEq g⁻¹ DW and remained unchanged for all NaCl concentrations. The roots relatively accumulated the same levels of potassium content for both NaCl-treated and untreated plants.

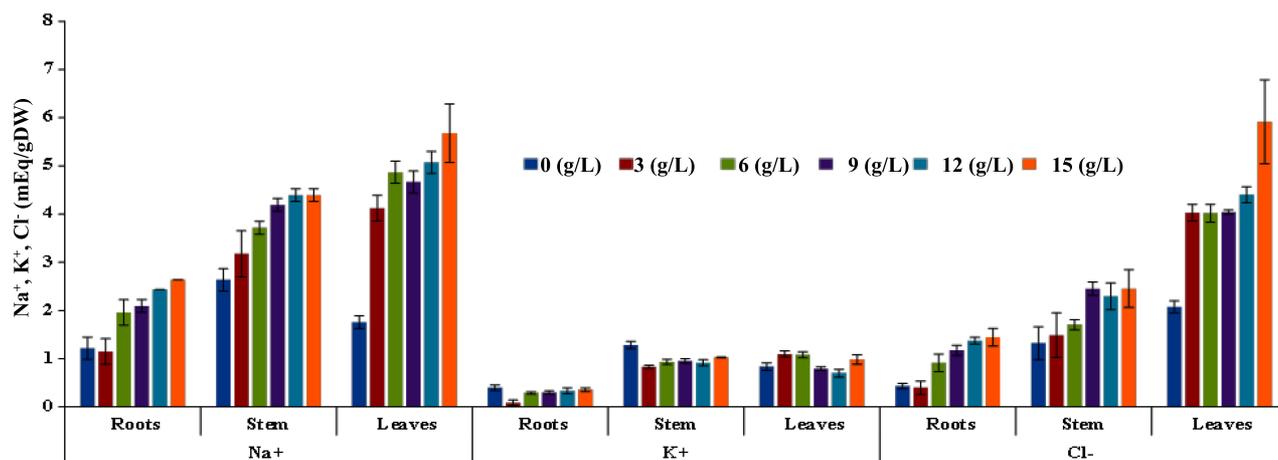


Figure 3. Effects of different NaCl concentrations on the ionic (Na⁺, Cl⁻, and K⁺) content of the different organs of kenaf plants.

3.3.4. K^+/Na^+ ratio

To further understand the competition between K^+ and Na^+ uptake and distribution within the aerial parts and roots, the K^+/Na^+ ratio was determined for all organs (leaves, stem, and roots) under the different NaCl concentrations (Figure 4). One of the key determinants of plant salt tolerance is the capacity of plants to maintain a high cytosolic K^+/Na^+ ratio. Under control conditions, the K^+/Na^+ ratio values were 0.5 and 0.35 for aerial parts (leaves and stems) and roots, respectively. Following NaCl treatment at any concentration, this ratio decreased by half in all compartments (leaves, stems, and leaves), showing that kenaf plants tend to accumulate more Na^+ than K^+ within the whole plant, indicating a high capacity of sodium detoxification via vacuolar compartmentalization. It noteworthy that the K^+/Na^+ ratios of leaves and stems were higher than those of roots, reasonably displaying a trait of tolerance.

The study of the effect of salt stress on plant growth was conducted by multivariate analysis, PCA. Regarding the PCA performed for NaCl treatments, by considering 12 parameters, the first two components (F1 and F2) explained 98.41% of the total variation. The first component (axis 1) explained 80.28% of the variation, followed by 18.13% for the second component (axis 2) (Figure 2).

The Spearman correlation matrix clearly showed that NaCl correlated positively with stem Na^+ ($R^2 = 0.945$), leaf Na^+ ($R^2 = 0.758$), root Na^+ ($R^2 = 0.967$), stem Cl^- ($R^2 = 0.896$), leaf Cl^- ($R^2 = 0.780$), and root Cl^- ($R^2 = 0.973$). Furthermore, it correlated negatively with leaf K^+/Na^+ , stem K^+/Na^+ , stem K^+ , and leaf K^+ ($R^2 = -0.775$, $R^2 = -0.742$, $R^2 = -0.516$, $R^2 = -0.407$, respectively).

These results were confirmed by two MLR models. The results from the MLR were obtained using NaCl, stem Na^+ , and stem K^+ as the dependent variables and stem K^+/Na^+ as the independent variable for the first model. Moreover, the second model was produced by means of NaCl, leaf

Na^+ , and leaf K^+ as the dependent variables and leaf K^+/Na^+ as the independent variable. These results revealed two major linear combinations of the constructs for predicting attitude. The obtained results show that 99.3% and 99.5% of the variance in model 1 and model 2, respectively, can be predicted using the independent variables.

This modeling analysis shows the standardized beta coefficients that present the contributions of each variable to the two models. The t- and P-values show the impact of the independent variables on the dependent variable. For the first model, all independent variables are insignificant except for stem K^+ , where $P = 0.016$. The large t-value ($t = 7.73$) and corresponding significant P-value ($P < 0.05$) support the results for insignificance, except for stem K^+ , which had the highest beta coefficient ($\beta = 0.413$). The value of the determination coefficient ($R^2 = 0.993$) indicates that 99.3% of the variability in the response could be explained by the first model. This result is confirmed by the ANOVA analysis for the estimated models of stem K/Na . As the P-value is 0.016, the model is significant. Thus, the combination of the variables significantly predicts the dependent variable ($F = 88.62$; $P < 0.05$). Similar results were shown for the second model.

3.4. Effect of salt stress on fiber yields

The fiber yields (NDF, ADF, and ADL) of kenaf plants cultivated in hydroponic conditions and under different NaCl concentrations are shown in Table 3. Interestingly, fiber yields from the Guangdong 743-2 variety are much higher than usual averages obtained from common cultivars (Akubueze et al., 2014). In addition, these yields were not significantly reduced by salinity. Up to 12 g L^{-1} NaCl, the NDF content remained similar to that under control conditions with around 80% of DW; it recorded a slight decrease only at 15 g L^{-1} NaCl with 75% of DW. Within the range of $0\text{--}6 \text{ g L}^{-1}$ NaCl, the ADF content was maintained to a value of 60% of DW, and it was slowly reduced to 55% of DW for $9\text{--}15 \text{ g L}^{-1}$ NaCl. However, the contents of ADL (37% of DW) were

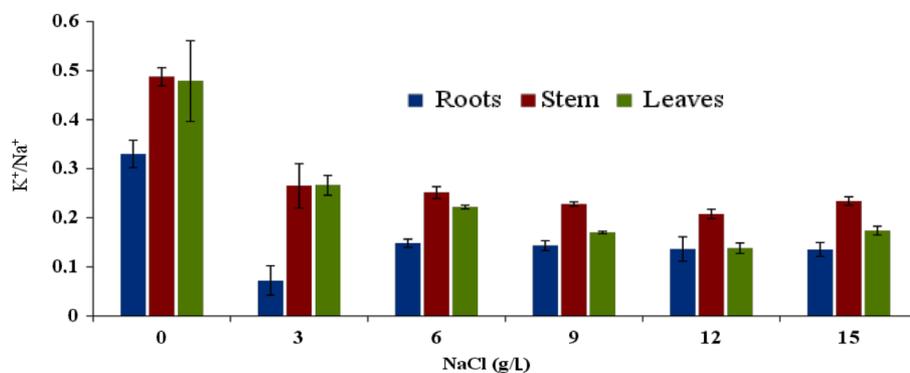


Figure 4. Effects of different NaCl concentrations on the Na^+/K^+ ratio of different organs of kenaf plants.

Table 3. Influence of different NaCl concentrations on fiber yields and components.

NaCl (g/L)	0	3	6	9	12	15
NDF (% DW)	79.71a	79.61a	78.18ab	76.17ab	78.01ab	74.05b
ADF (% DW)	56.41a	58.13a	56.77a	50.05b	50.91b	50.02b
ADL (% DW)	32.97a	31.40a	35.50a	37.64a	32.22a	31.02a
Hemicellulose (%)	23.3AB	21.5B	21.4B	26.1AB	27.1A	24.0AB
Cellulose (%)	23.5A	26.7A	21.3A	21.4A	18.7A	19.0A
Lignin (%)	33.0A	31.4A	33.5A	37.6A	32.2A	31.0A

NDF: Neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin. Values with the same letter in the same line are not significantly different ($P \leq 0.05$).

invariable regardless of the salt concentration. The contents of hemicellulose, cellulose, and lignin shown in Table 3 were calculated from the values of fiber contents. The obtained results of these components' contents accentuate the fact that none of them were changed or affected by salinity. Interestingly, the values of hemicellulose and cellulose remained the same, around 20%–25% for all treatments, irrespective of salinity, similarly to those of lignin around 35%. The different NaCl concentrations applied to kenaf plants were found to have no influence on the production of fiber components, showing that kenaf plants are able to produce and maintain their fibers (NDF, ADF, and ADL) and components of fibers (hemicellulose, cellulose, and lignin) even under high levels of salinity.

The study of the effect of salt stress on fiber yields was completed by multivariate analysis, PCA. Regarding the PCA performed for NaCl treatments, by considering 6 parameters, the first two components (F1 and F2) explained 98.05% of the total variation. The first component (axis 1) explained 74.71% of the variation, followed by 23.34% for the second component (axis 2) (Figure 2). In the same way, the Spearman correlation matrix clearly showed that NaCl was correlated positively with hemicellulose ($R^2 = 0.855$) and negatively with ADF, NDF, and cellulose ($R^2 = -0.891$, $R^2 = -1.000$, and $R^2 = -0.636$, respectively).

4. Discussion

In the Mediterranean basin, salinity is one of the major constraints impeding germination and affecting seedling and plant growth (Gupta and Huang, 2014). The utilization of kenaf as a salt-tolerant industrial crop would be useful to recover salinized land and to valorize its fiber components for numerous industrial applications (paper and pulp, fabrics, textiles, biocomposites, insulation mats, absorption materials, animal bedding, etc.). Since it is impossible for forests to produce an annual quantity of fiber to meet domestic demands, nonwood products have become one of the major alternative sources of fibrous materials for the

21st century. Kenaf represents an alternative crop that may be a feasible source of economically viable and ecologically friendly cellulose.

Salt stress adversely affects plants at all stages of their life cycle, but many plants are most sensitive to salt during seed germination. However, seeds may be more sensitive to stresses than mature plants because of exposure to the dynamic environment close to the soil surface. Seed germination is known to be a critical point in seedling establishment, subsequent plant vigor, and ultimately the obtaining of successful crop production.

Furthermore, the phenotypic response of seeds to salinity could also be an indicator of plant behavior for the later stages of development. Even if there have been some debates as to whether kenaf avoids, tolerates, or escapes osmotic stress, currently there are limited data about its effect and influence on kenaf seed germination (Curtis and Lauchli, 1985, 1986; Jin et al., 2012). Therefore, our study was conducted as a first attempt to assess the germination behavior of kenaf under salt stress in order to plan, in a further attempt, its introduction for field cultivation. As previously mentioned, since germination is an important physiological stage of the growth cycle of the plant and the installation of future plants, our study started with the assessment of the level of salinity tolerance of kenaf seeds at the germination phase. Thereafter, multivariate analyses (PCA and MLR) were used to evaluate the salt tolerance of kenaf. These statistical methods have numerous advantages. PCA allows a simultaneous analysis of several parameters to elevate the accuracy of the plant status at different salt levels. Moreover, MLR is an excellent tool to predict whether a model shows interactions between parameters. It appeared that kenaf seeds were able to germinate at up to 15 g L^{-1} NaCl (40%), and with a notable rate (75%) at 12 g L^{-1} NaCl. Similar results obtained by Curtis and Lauchli (1985) showed that kenaf seed germination was slightly affected by 200 mM NaCl ($\sim 12 \text{ g L}^{-1}$ NaCl).

Although kenaf behaves as a halophyte at the germination phase, germinating seeds and young seedlings of halophytes are sensitive to salt starting from concentrations higher than 200 mM NaCl, despite their high tolerance as adults (Panuccio et al., 2014; Flowers et al., 2015; Slama et al., 2015). In addition, kenaf seeds were able to germinate very fast, even under saline conditions. In order to obtain fast and good establishment of seedlings, high vigor seed is needed to provide essential nutrients for seedlings until they become established and able to photosynthesize independently (Bewley and Black, 1994). The falling of leaves, mainly older ones, is a common symptom for osmotic stress. Indeed, according to Munns (2002), the accumulation of sodium chloride in the cytoplasm may be responsible for their fall. Ionic stress results in toxicity symptoms (chlorosis, necrosis) in mature leaves followed by the senescence of older leaves due to high Na⁺ and Cl⁻ content, thus reducing the photosynthetic capacity of the plant (Carillo et al., 2011).

Plant growth inhibition and yield reduction are the general effects of salinity. The research works of Curtis and Lauchli (1985, 1986) on some kenaf varieties (Cuba-108, Guatemala, Everglades 45 and 71) showed a reduction in biomass under 75 mM NaCl treatment. In comparison with these previous varieties, we found better results and behavior with the Guangdong 743-2 kenaf variety, for which no significant growth inhibition was observed at up to 15 g L⁻¹ NaCl (~250 mM), yet with leaf DW reduction starting from 6 g L⁻¹ NaCl (~100 mM). The root growth of Guangdong 743-2 is favored over leaf growth during the early stages of salt stress; thus, the growth of the organ exploiting the most limiting resource (i.e. water) is favored (Hsiao and Liu-Kang, 2000). In this context of salt-tolerant variety selection using the inhibitory effect of salt stress on growth as the main criterion of selection, Jin et al. suggested Tainung-2 as a potential cultivar for popularization and farm cultivation (Jin et al., 2012). However, these results are not very informative about the physiological mechanisms of salt tolerance and data are still missing about salinity's impact on fiber yields. Since the morphological symptoms in response to salinity may not be enough to determine its effect, it is necessary to analyze other physiological parameters, including ionic homeostasis and water status. In our investigations of kenaf's behavior under salt stress, water content, ionic distribution (namely Na⁺, K⁺, and Cl⁻), fiber yields, and fiber component contents were therefore assessed and analyzed to give a comprehensive understanding of how kenaf variety Guangdong 743-2 responds to and copes with salt stress. Multivariate analyses based on these parameters proved to be valuable methods for the study of salt tolerance. Our results showed a variation of 98.41% by studying the effect of salt stress on mineral nutrition (Figure 2). Spearman correlation analysis also showed clearly that NaCl was correlated positively with Na⁺ and

Cl⁻ ionic distribution in roots, stems, and leaves of the studied plants.

Usually, under salinity constraint, the water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress (Gupta and Huang, 2014). In the present case, kenaf variety Guangdong 743-2 was able to maintain its root WC steady in reaction to salt stress, but not for its leaves and stems, whose WC decreased very fast. The decrease in leaf WC would be responsible for their fresh weight decline, wilting, and ultimate falling. Although roots are directly exposed to salt, they proved to be surprisingly robust. As previously shown, their growth rate and WC were not as affected as those of leaves. Kenaf plants have likely evolved protective mechanisms that keep favorable WC within their roots, thus minimizing osmotic stress. Moreover, Guangdong 743-2 shows an ability to regenerate new leaves during its growing period even under salt stress, relatively reestablishing the photosynthetic activity. This is confirmed by the Spearman correlation coefficient, which shows that NaCl is negatively correlated with leaf WC ($R^2 = -0.983$).

Initially, NaCl stress is known to repress plant growth in the form of osmotic stress that is then followed by Na⁺ and Cl⁻ toxicities (Carillo et al., 2011). The accumulation of Na⁺ and Cl⁻ within plant tissues is the most detrimental effect of salinity stress that leads to nutritional deficiencies and imbalances as well as dehydration and enzyme activity inhibition (Gupta and Huang, 2014). Almost nothing is known about sodium and chloride detoxification in kenaf. The results of mineral composition analysis demonstrated that the sodium and chloride accumulations increased with the increase in salt levels within the whole plant and mainly in the aerial parts, while potassium contents decreased, but with an irregular increase in leaves. The higher Na⁺ and Cl⁻ that accumulated in the whole plant would be used as a cheap osmoticum in the vacuole. Plants cope with high NaCl concentrations via the reduction of NaCl entry into roots, salt ion exclusion from the shoots, or salt ion compartmentalization inside the vacuoles (Jha et al., 2010). Salt-tolerant plants accumulate higher Na⁺ concentrations in vegetative tissues than sensitive ones, which is indicative of greater Na⁺ homeostasis capacity (Flowers et al., 2015). Guangdong 743-2 behaves as an includer species, which consists of sodium chloride accumulation within shoots and leaves, a strategy achieved by ion compartmentalization in the vacuole via sodium/proton antiporters (Hanana et al., 2007; Bassil and Blumwald, 2014; Reguera et al., 2014) and chloride channels (Flowers et al., 2015; Nguyen et al., 2016). Hence, the excess of salt is either transported to the vacuole or sequestered in older tissues that eventually are sacrificed, thereby protecting the plant from salinity stress, and in the meantime new leaves are synthesized to ensure photosynthetic activity.

As a consequence of the pattern of ion accumulation within kenaf tissues, the K^+/Na^+ ratio diminished in all organs. Both glycophytes and halophytes need to keep an optimal Na^+/K^+ ratio in a metabolically active cytosolic compartment. In general, although NaCl treatment reduces the total K^+/Na^+ ratio, it may be misleading as a criterion for salt tolerance, since Na^+ could be compartmentalized inside vacuoles. The regulation of this ratio and ionic homeostasis establishment depend on many transport steps involved in the uptake, efflux, translocation, and compartmentalization.

Despite the severe NaCl treatments applied to the kenaf plants, fiber yields and their components remained steady, demonstrating the good adaptation of kenaf.

The present findings allow us to affirm that kenaf variety Guangdong 743-2 is a NaCl-tolerant variety that, at both germination and the vegetative stage, was able to withstand high NaCl concentrations. Indeed,

Guangdong 743-2 seeds could germinate promptly and plants were able to maintain root and stem biomass production and preserve their root WC under salinity constraint. Moreover, the osmotic adjustment to NaCl salinity was conferred by inorganic ion accumulation in vegetative tissues, especially Na^+ and Cl^- , via their compartmentalization inside the vacuole, therefore adopting a strategy of ion inclusion. Remarkably, although salinity reduced the shoot biomass, fiber yields and fiber component production were kept unchanged. Thus, kenaf shows potential utility as a promising industrial crop in salty lands.

In conclusion, kenaf variety Guangdong 743-2 with high fiber yields and exceptional salt tolerance behavior could be useful to recover salinized land, increase the poor agricultural economy of the marginal regions of the Mediterranean area, and ultimately open new and promising opportunities for the kenaf agroindustry.

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