Germination potentiality of kenaf seeds under osmotic stress

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ABSTRACT

The increasing demand for wood production led to the decline of Tunisian forest area. Thus, culture of kenaf (Hibiscus cannabinus L.) could be an interesting and useful alternative in Tunisia to meet the industrial needs in fiber and cellulose for pulp and paper making. Yet, abiotic stresses, such as drought are serious threats to agriculture. Moreover, dehydration is one of the most important abiotic factors limiting plant germination. Indeed, germination of seeds, the most critical phase of plant life, is greatly influenced by water availability. However, few works have been made to assess and describe kenaf responses and behavior versus environmental stresses and in particular, no study has been made in relation to the effect of drought stress on kenaf seed germination. In order to propose the introduction of kenaf cultivation, it is essential to test its behaviour under agro-climatic conditions of Tunisia. Thus, and before the evaluation of the yield of this valuable industrial crop, it is important to assess, in a first attempt, the response of kenaf under drought stress at the germination stage. Therefore, our research aims to evaluate the germination potentiality of kenaf seeds under osmotic stress. Kenaf seeds were disinfested for cultivation under stressful osmotic potentials (0, -2, -4, -6, -8 and -10 bars), respectively induced by different concentrations of polyethylene glycol (PEG-6000) in order to assess their behaviour under dehydration conditions. Kenaf seeds displayed interesting behaviour under drought constraint and showed high ability of germination under severe osmotic stress. Indeed, they could germinate until -6 bars at a rate of 77%. Thus, kenaf could be classified as drought tolerant species during its germination stage and theoretically, would not find difficulty of cultivation installation in our pedo-climatic conditions.

Key words: Kenaf, Hibiscus Cannabinus L., germination, osmotic stress, polyethylene glycol.

INTRODUCTION

Kenaf (Hibiscus cannabinus L.) is a warm season annual crop belonging to the Malvaceae family (Paul et al., 2002) that grows in tropical and temperate climates and thrives with abundant solar radiation and high rainfall (Coetzee et al., 2008). Kenaf produces up to 30 t/ha of dry stem material (Wood, 2003) and yields approximately three to five times as much fiber as southern pine (LeMahieu et al., 2003). The traditional use of kenaf focuses on its fiber production (Li, 1980). New applications of kenaf such as pulping papermaking and board making were recently developed; it has also been identified as an excellent source of cellulosic fiber for the manufacturing of a large range of paper products (Bhardwaj et al., 2005).

In Tunisia, the 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 (Ghobtane, 2010), Esparto...
pulp production decreased (SNCPA, 2002; Belkhir et al., 2013). The introduction of kenaf in Tunisia can represent a renewable and promising source of natural cellulosic fiber and material for the paper industry. However, little or none is known about its seed production and germination ecophysiology in Tunisia.

Kenaf is introduced into arid regions and is increasingly being grown in other dry, light-textured marginal soils with the probability of water deficits developing during the growth period (Francois et al., 1992). The adverse effects of water deficit on growth and metabolism are well known. Water deficit affects many plant species and drought stress is a critical factor limiting crop production even in the rainy season for soils in the humid and sub-humid tropics (Ogbonnaya et al., 1998). While kenaf is broadly adapted, many researchers stated that soil and environmental differences between locations preclude the transfer of knowledge from one location to another and each recommending testing wherever there is interest in growing kenaf to determine its suitability for that region (Muchow et al., 1990; Francois et al., 1992; Kemble et al., 2002).

Water deficit is one of the most important environmental stresses affecting agricultural productivity around the world (Soltani et al., 2006; Slama, 2007). Current estimates indicated that 25% of the world’s agricultural lands are now affected by water stress (Jajarmi, 2009). For Tunisia which is located on the southern part of the Mediterranean basin and in the north of Africa, drought is probably the most feared phenomenon of the expected climatic change during the XXI century (Nasr et al., 2009).

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri et al., 2001; Hamrouni et al., 2012). Studies on seed germination of a species in conservation biology are important due to assessment of the adaptation of its seed germination and seedling emergence which may not only contribute to the knowledge of its reproductive strategy and population regeneration, but it is also critical to the conservation and management of the species (Yang et al., 2008).

Furthermore, the phenotypic response of seeds to drought could also be an indicator of plants behavior for the later stages of development (Radhouane, 2007). One technique for studying the effect of water stress on germination is to simulate stress conditions using artificial solutions to provide stressful water potentials (Sharma, 1973; Falusi et al., 1983). Polyethylene glycol (PEG) is a non-ionic water polymer widely used to induce water stress, because it is not expected to penetrate into plant tissue (Kawasaki et al., 1983). Even if there have been some debates as to whether kenaf avoids, tolerates or escapes drought (Ogbonnaya et al., 1998), currently, there are few data about the effect of osmotic stress on kenaf seed germination.

Therefore, our study was conducted to assess in a first attempt the germination behavior of kenaf under drought stress, in order to plan, in a further attempt, its introduction for field cultivation.

**MATERIALS AND METHODS**

**Germination**

Kenaf seeds of Chinese variety, ‘Guangdong 743-2’ were used for this analysis. They were first surface sterilized for 30 min by fungicide (Pelt 44, 1 g/L) and then washed thrice with sterilized distilled water. Twenty seeds of kenaf were cultivated in each plastic Petri dishes containing sterile perlite moistened with 15 ml of respective PEG$_{6000}$ concentrations for each osmotic potential treatment, including a control treatment with five (5) replications. The Petri dishes were covered to prevent the loss of moisture by evaporation and then placed in a growth chamber at 25 ± 1°C under continuous light. Number of germinated seeds was daily counted and data were recorded for 10 days. Seeds were considered germinated when the emergent radicle reached 2 mm length.

**Osmotic stress**

Osmotic solutions of PEG are commonly used to control water potential in seed germination studies. The different osmotic potentials treatments are managed using PEG molecules with a M$_r$ ≥ 6000 (PEG$_{6000}$). This latter biochemical compound was used because it is inert, non-ionic and its virtually impermeable chains induce water stress and maintains uniform water potential throughout the experimental period (Hohl and Peter, 1991; Lu and Neumann, 1998). Moreover, molecules of PEG$_{6000}$ are small enough to influence the osmotic potential, but large enough not to be absorbed by plants (Carpita et al., 1979; Saint-Clair, 1980). Since PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimicked dry soil more closely than solutions of low M$_r$ osmotica which infiltrate the cell wall with solutes (Veslues et al., 1998). PEG has been more effective in research works, as it does not penetrate the cells, not degraded and does not cause toxicity due to its high molecular weight (Hasegawa et al., 1984; Silva et al., 2001).

The osmotic potential of the aqueous solutions of PEG$_{6000}$ was calculated according to the empirical equation developed by Michel and Kaufmann (1973). Water stress was applied through incubation in five different concentrations of PEG$_{6000}$ providing solutions with water potentials ranging from -2 to -10 bars (Table 1). PEG$_{6000}$ was prepared by dissolving the required amount of PEG in distilled water and placed in a shaker at room temperature, while distilled water was used as the control.
Table 1: Concentrations of PEG6000 with corresponding osmotic potentials.

<table>
<thead>
<tr>
<th>Osmotic potential (bar)</th>
<th>PEG6000 (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-2</td>
<td>112.23</td>
</tr>
<tr>
<td>-4</td>
<td>169.11</td>
</tr>
<tr>
<td>-6</td>
<td>213.63</td>
</tr>
<tr>
<td>-8</td>
<td>251.02</td>
</tr>
<tr>
<td>-10</td>
<td>284.02</td>
</tr>
</tbody>
</table>

Calculations

From the germination collected data, the following variables were calculated.

*Germination ability

Cumulative percentage germination (%)

This is the ratio of the number of germinated seeds on the total number of seeds per sample (Yang et al., 2008).

Germination velocity coefficient (CV)

This was calculated from daily germination counts based on the formula of Kotowski (1926) given as:

\[
CV = 100 \times \frac{n_1 + n_2 + \ldots + n_x}{(n_1 t_1 + n_2 t_2 + \ldots + n_x t_x)}
\]

Where, \(n_1, n_2, \ldots, n_x\) number of seedlings counted on the first and second day, etc until the last day (x), and \(t_1, t_2, \ldots, t_x\) number of days between sowing and the first collection and between the sowing and the second collection, etc until the last collection (x) (Rannal et al., 2006).

*Germination kinetic

A simple theoretical model was used to analyze the germination kinetic. This model assumes that the germination process comprises of two steps: a phase of latency, during which the seeds acquire the aptitude to germinate and followed by the germination itself. After the latency, the probability of germination per unit of time is the same for all the seeds and constant with time (Debez et al., 2004). The total percentage of germination and time required to reach 50% of germination based on the total number of seeds was determined (Hardegree et al., 2002; Clua et al., 2006). According to Brown et al. (1988) and Essemine et al. (2007), the time required to achieve 50% of germination was calculated by interpolation from the cumulative germination curve.

Statistical analysis

Germination data were analyzed using the statistical SAS program for the analysis of variance method (ANOVA) (SAS Institute, 1989). The differences between means were tested for significance using Student–Newman–Keuls test at 5% significance level. The experiment was designed as a completely randomized design (CRD) with one factor of the drought stress treatments.

RESULTS

Effect of osmotic stress on germination rate

Figure 1 shows the effect of various osmotic potentials on germination percentage. Osmotic treatments led to a decrease in the germination percentage. However, from 0 to -4 bars, no significant reduction in seeds germination was registered. At -6 bars, germination of kenaf seeds decreased to a rate of 77%, which is still considered a high value for this osmotic potential. However, at -8 bars, there was a drastic fall in the germination percentage with 8%. Finally, germination was totally inhibited at -10 bars treatment.

Analysis of variance was used to test for differences between the treatments. Mean germination rates comparison did not reveal significant difference (p<0.05) between 0, -2 and -4 bars osmotic potential levels (Table 2). Moreover, -4 and -6 bars germination rates were considered similar. In comparison with the control condition (0 bar), germination rate at -6bars was reduced only by 21% (Figure 1).

Effect of osmotic stress on germination kinetic

Table 3 shows that coefficient of velocity follows the same pattern as germination percentage. They both decreased due to the increase of PEG6000 concentration. However, there was no significant difference between -6 and -8 bars for the CV. Kotowski (1926) reported that the value of the coefficient of velocity increased when the number of seedlings increased.

Figure 2 shows the kinetics of germination under
Figure 1: Germination percentage of kenaf seeds under different osmotic potentials. Values with different letters are significantly different (P>0.05).

Table 2: ANOVA of germination rate values.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of square</th>
<th>DF</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG concentration</td>
<td>49427.50</td>
<td>5</td>
<td>9885.50</td>
<td>132.54</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1790.00</td>
<td>24</td>
<td>74.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>51217.50</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Germination percentage and coefficient of velocity of kenaf seeds under different osmotic potentials.

<table>
<thead>
<tr>
<th>Osmotic potential</th>
<th>Germination percentage</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 bar</td>
<td>97a</td>
<td>94.37a</td>
</tr>
<tr>
<td>-2 bars</td>
<td>96a</td>
<td>89.39a</td>
</tr>
<tr>
<td>-4 bars</td>
<td>86ab</td>
<td>91.49a</td>
</tr>
<tr>
<td>-6 bars</td>
<td>77b</td>
<td>58.40b</td>
</tr>
<tr>
<td>-8 bars</td>
<td>8c</td>
<td>50b</td>
</tr>
<tr>
<td>-10 bars</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values with different letters are significantly different (P>0.05).

osmotic stress conditions. The values shown are the percentage of germinated seeds determined at the indicated days at different osmotic potentials (Figure 2). The increase in osmotic stress conditions induced variations not only in the germination ability but also in the mean germination time. Figure 2 shows that germination kinetic follows a sigmoidal pattern under control condition and -2 and -4 bars with 3 predominant phases:

1) A latent phase where seeds start imbibitions;
2) An exponential phase where seeds start germinating and
3) Finally, a plateau phase corresponding to the end of germination.

Both control, -2, -4 and -6 bars treatment seeds started germinating from the second day of sowing at different germination rates, while -8 bars treated seeds started their germination following the third day of sowing with a very low percentage of germination and directly entered the plateau phase (Figure 2). Control, -2 and -4 bars treated seeds reached their maximum rate of germination at the second day, while -6 bars treated seeds reached the top of
germination at the third day. Mean time of 50% of germination was less than two days for control, -2 and -4 bars treated seeds, while it took two days and a half for -6 bars treated seeds. This delay of germination shows the lowering of the germination speed due to the osmotic stress.

DISCUSSION

The first imperative of a satisfactory culture consists of its suitable establishment; consequently, the success of the germination phase determines the vegetative period. Furthermore, the answer of seeds to drought could also be an indicator of the tolerance of plants for the later stages of development. The study of the influence of the drought using osmotic solutions is one of the common methods used to analyze the seeds behavior during the germinal phase (Radhouane, 2007). In the present experiment, the effect of osmotic potential on seed germination of *H. cannabinus* was studied.

Drought stress treatment applied within germination media affected both percentage and speed of seed germination at the lowest osmotic potential. Indeed, a number of studies have confirmed that seeds of different plants show dissimilar germination capacity under water limiting conditions (Ibanez and Passera, 1997; De Villalobos and Pelaez, 2001; Joel and Oscar, 2001; Zeng, 2010) by decreasing the percentage of germination seeds (Delachiave et al., 2003) and delaying its beginning (Hardegree and Ermerich, 1990). These differences originate from natural diversity and have special eco-adaptation significance for each species.

In our study, the highest germination percentage was recorded in the control treatment (98%) as against a value of 8% at -8 bars. Although, the osmotic stress induced by PEG decreased seed germination capacity, however, at a very low potential (that is, -6 bars), a value of 77% of germinated seed was recorded indicating its high tolerance to drought stress. Moreover, seeds cultivated under the control conditions are faster to germinate than those treated by -8 bars. In this context, Gholami et al. (2010) proved that PEG not only delays the germination but also affects the final germination percentages. Our results are not in agreement with those reported by Almansouri et al. (2001) who stated that moderate osmotic stresses only delay germination while high stresses reduce the final germination percentages. According to Ben et al. (1986), this delay would be explained by the time necessary to start the necessary mechanisms of seeds for adjusting their osmotic potential.

In previous report, Ogbonnaya et al. (1998) found that Cuba 108 variety of kenaf have a high tolerance capacity to water deficit. This might be due to membrane resistance and/or osmotic adjustment mechanisms triggered when water deficit surpasses the critical point of -0.5 MPa. In contrast, the osmotic stress led to a reduction of the percentage of germination to 48% at -2 bars as compared to the control (Dirik, 2000). In the same context, Radhouane (2007) proved that the seeds of *Pennisetum glaucum* were affected by the osmotic stress at relatively low potential.

Although kenaf is a high water-demanding crop (Amaducci et al., 2000), water stress is not always injurious since it sometimes improves the quality of plant products (Patanè et al., 2010). In spite of the high temperature and high moisture being favorable for
growth, kenaf tolerates drought (Banuelos et al., 2002). It also performs well in warm, dry conditions when precipitation is adequate (Ching et al., 1993). Having a prolific root system with a long taproot and wide-ranging lateral roots allows kenaf to be responsive to changes in soil moisture (Stricker et al., 1997; Lauriaulta et al., 2009).

Conclusions
In this study, drought tolerance in early phase of kenaf seedling growth was investigated by evaluating germination process under PEG induced osmotic stress. Based on the results of this study, it was concluded that the seeds can germinate at very low osmotic potential level with high rate (77%). It was observed that osmotic stress delays germination of seeds treated by -8 bars. We therefore, suggest that kenaf can tolerate drought stress at germination stage. However, field studies are needed to study its yield and fiber production and to determine their suitability for areas with specific environmental conditions. Under natural conditions, seed germination is more complicated and influenced by several factors such as salinity, drought, light and temperature.

It is therefore recommended that future studies focus on the interactive effects of these factors to better understand the ecophysiological strategies of plants for survival under natural environmental conditions.

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