academicJournals

Vol. 9(1), pp. 65-73, 2 January, 2014
DOI: 10.5897/AJAR11.116
ISSN 1991-637X ©2014 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Germination responses of *Corchorus olitorius* L. to salinity and temperature

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Accepted 4 December, 2013

In order to investigate the effect of temperature and salinity on the germination of the wild leaf vegetable *Corchorus olitorus* L., seeds of two varieties of *C. olitorus* from ´Bou Salem´ and ´Menzel Bouzelfa´ responses were determined in complete darkness over a wide range of temperatures and salinities. The final germination percentage (FGP) and mean time to germination (MTG) were measured. Germination was inhibited by either an increase or decrease in temperature. For the two varieties, optimal germination was at 30°C. Highest germination percentages were obtained under no-saline conditions. The final germination percentage and germination rate (estimated by MTG) significantly decreased with increasing salt. This study has shown a high degree of variation of these characters mainly related to geographical origin. It was observed also that ´Menzel Bouzelfa´ variety was less affected by the imposed salt stress than ´Bou Salem´ variety.

Key words: *Corchorus olitorus*, germination responses, salinity, temperature.

INTRODUCTION

Corchorus belongs to the family Malvaceae and genus *Corchorus* consists of some 50 to 60 species of which about 30 are found in Africa (Emongor et al., 2004). *Corchorus* is mainly known for its fibre product jute and for its leaf vegetables (Imbamba, 1973). Jut is mainly extracted from *C. olitorius* and is most frequently cultivated. *C. olitorius* is a large cosmopolitan tropic. It is a green leafy vegetable that is rich in betacarotene, iron, calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol equivalent Vitamin E (Leung et al., 1968). *C. olitorius* is an erect annual herb that varies from 20 cm to approximately 1.5 m in height depending on the cultivar. The stems are angular with simple oblong to lanceolate leaves that have serrated margins and distinct hair-like teeth at the base. The bright yellow flowers are small and the fruit is an angular capsule. The wild forms of *C. olitorius* grow in the savannah, fallow or abandoned fields, often near swamps, rivers and lakes, at altitudes up to 1250 (-1750) m. *C. olitorius* grows best in hot and humid climates where annual rainfall is between 600 to 2000 mm, and it prefers sandy loam soils rich in organic matter and pushes hard on the heavy clay (Edmonds, 1990). *C. olitorius* seeds show a high degree of dormancy which can be broken by means of hot water treatment (Schippers et al., 2002a). It has been recorded to establish naturally from seeds and tolerates a wide range of soils and climates (Oladiran, 1986).

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress (Chapman, 1974; Ungar, 1995), the mechanism(s) of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and

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biochemistry in vegetative plants (Hester et al., 2001; Garthwaite et al., 2005; Hu et al., 2005; Ren et al., 2005; Kanai et al., 2007). Salinity affects seed germination through osmotic effects (Bliss et al., 1986), ion toxicity (Hampson and Simpson, 1990) or a combination of the two (Huang and Redmann, 1995). Plants can be classified into two main groups based on their response to saline stress, salt-tolerant halophytes and salty-tolerant glycophytes. However, this classification is somewhat artificial as the implied discreteness of response does not exist in reality, with responses occurring along a gradient (Greenway and Munns, 1980). Salinity-induced reduction in the germination of halophytes is mainly due to osmotic effects only, whereas glycophytes are more likely to exhibit additional ion toxicity (Dodd and Donovan, 1999). The high environmental salinity inhibits germination, the detrimental effects of salinity are generally reduced at optimum temperatures (De Villiers et al., 1994; Aiazzi et al., 2002; Khan et al., 2002), with decreased germination noted in various species at either supra- or sub-optimal temperatures (Gulzar et al., 2001; Khan and Gulzar, 2003). Decreases in germination have been attributed to increased evaporation of moisture at high temperatures, increasing salt concentration by capillary movement (Khan and Ungar, 1996). Salinity–temperature interactions may have significant eco-physiological implications in terms of germination rates and date under field conditions (Ungar, 1995). Tolerance to salinity during germination could be decisive for the establishment of plants growing in saline soil (Ungar, 1995; Khan and Gulzar, 2003). In this way, the knowledge of thermal requirement and the salinity tolerance of seeds is subsequently necessary; particularly with the possible utilization of this species in medicinal and in commerce. The aim of this study was to determine optimal germination temperature of two *C. olitorus* varieties and to investigate the germination responses to salinity.

MATERIALS AND METHODS

Two varieties of *C. olitorius* were used: 'Bou Salem' (sub-humid) and Menzel Bouzelfa (huper semi-arid). Seeds were stored in a cool, dark room prior to the start of the experiment. The seeds in both samples were approximately 18 months old when the experiments were carried out (April 2011). Approximately 100% of 'Bou Salem' and 'Menzel Bouzelfa' seeds were germinable.

Germination experiments

Germination studies were conducted in incubators with a 12-h photoperiod (Sylvania cool white fluorescent lamps, 25 mmol photons m^{-2} s^{-1} photosynthetically active radiation). In an attempt to remove germination inhibitors, the seeds were immersed in hot water 100°C for 20 min. Seeds were then surface sterilized in 0.58% sodium hypochlorite solution for 1 min, washed with distilled solution were prepared. Seeds were germinated in the dark in Petri dishes of 9 cm diameter fitted with two layers of Whatman No. 1 filter paper moistened initially with 5 ml of distilled water. Each treatment consisted of four replicates with 25 seeds. Germination experiments were conducted in incubators set at 15, 20, 25, 30, 40 and 45°C in darkness (Lumінincube II; Analys, Belgium; MLR-350, Sanyo, Japan). Seeds were germinated in 0, 50, 100, 150 and 200 mmol NaCl solutions under the optimal temperature of germination and in dark treatments. Germinated seeds were counted and removed every second day over a period of 16 days (Gulzar et al., 2001; Khan and Gulzar, 2003; El-Keblawy and Al-Rawai, 2005). Seeds were considered germinated when radical appeared (Redondo-Gomez et al., 2007). Two germination characteristics were determined: final germination percentage (FGP) and mean time to germination (MTG). MTG was Nichols and Heydecker, 1968) was calculated as follows:

$$MTG = \sum_i (n_i \times d_i)/N$$

where n_i is the number of seeds germinated at day i, d_i is the incubation period in days, and N is the total number of germinated seeds (Brenchely and Probert, 1998; Redondo-Gomez et al., 2007).

Statistical analysis

The original data - the number of germinated seeds per sample was transformed so that distribution will be normal and the variances homogeneous. The transformation used was the arcsine of the square root of the proportion of germinated seeds per sample (Jozef et al., 2003). Germination characteristics data were analyzed using appropriate procedures of the SAS software 6.12 (version 1998). Analysis of variance (ANOVA) was performed with the statistical program Minitab (Minitab Inc., College Park, PA), involving two levels of classification (salinity and population) with interactions. A Duncan's multiple range tests was carried out to determine if significant (P < 0.05) difference occurred between accessions and treatments.

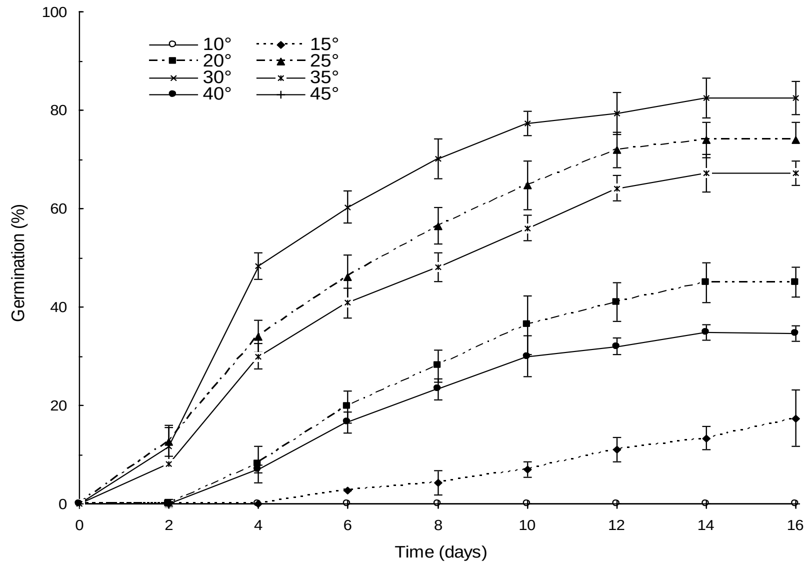
RESULTS

Optimal germination temperature

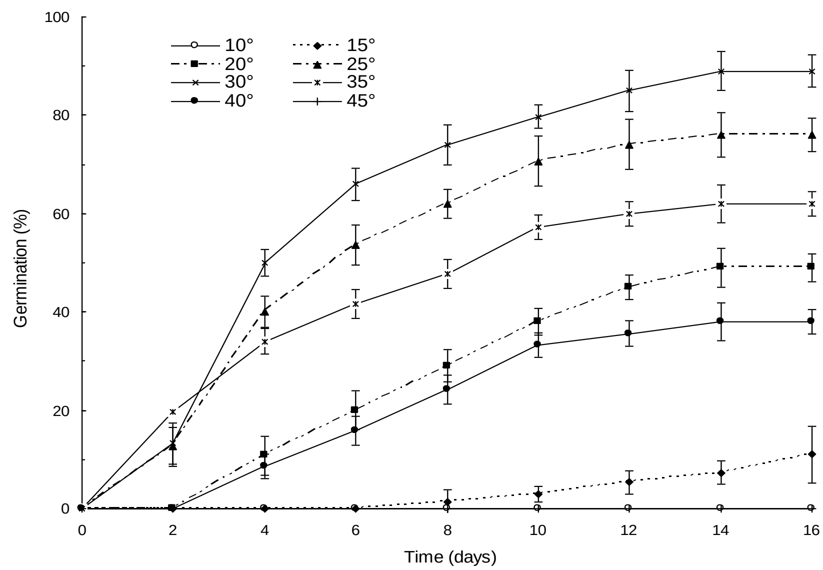
Figure 1 shows that two *C. olitorius* varieties ´Bou Salem´ and ´Menzel Bouzelfa´ seeds can germinate under wide range of temperature. In distilled water, the seeds germinated rapidly in the range 25 to 35°C. The optimal temperature of germination was 30°C and the maximum percentage (83 and 90% for ´Bou Salem´ and ´Menzel Bouzelfa´ respectively) was achieved after two weeks (14 days). Germination was completely inhibited at 15, 20 and 40°C. However, at 10 and 45°C no seeds germinated for the two *C. olitorius* varieties. Germination commenced from the 4th day at 20 and 40°C and from the 6th day at 15°C. The MTG decreased with increasing temperature until 30°C (30°C, MTG was only a half of that recorded at 15°C (data not presented). However, up to 30°C, the opposite was observed. Hence, 30°C was chosen for the study of seeds germination responses in the two *C. olitorius* varieties.

Salinity tolerance

Variance analysis revealed a highly significant population and treatment effects (Table 1). Indeed, FGP showed



(a)



(b)

Figure 1. Cumulative germination percentage of tow *C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa seeds incubated for 16 days at different incubation temperature (10 to 45°C). The data are means values of four measurements. The confidence intervals were calculated at the threshold of 5%.

significant difference ($p < 0.005$) indicating a height level of genetic variability. But, no significant population effect was detected for MTG. the two parameters (FGP and MTG) were significantly affected by salt treatment ($p < 0.005$). Population x treatments interactions were also affected FGP and MTG ($p < 0.005$).

Germination of the *C. olitorius* varieties was significantly ($p < 0.005$) inhibited by NaCl concentration higher than50 mM (Table 1 and Figure 4). The effect of salinity on FGP was similar in two varieties. Indeed, the highest germination percentages were found in distilled water (83 and 90% for ´Bou Salem´ and ´Menzel Bouzelfa´ respectively), followed by 50 mM (66 and 72% for ´Bou Salem´ and ´Menzel Bouzelfa´ respectively). Higher NaCl concentration (150 and 200 mM NaCl) showed substantial reduction in seed germination (Figure 2). *C. olitorius* varieties showed the highest germination percentage in the non-saline control treatment. At 50 mM NaCl, it showed slightly lower germination percentage (Figure 3) under high salinity (100 mM or more), this

Table 1. Analysis of variance (two-way ANOVA) and Comparison of the mean (Duncan test) of germination characteristics of tow *C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa seeds in five salinity treatments at during 16-day period.

Population	Traitement	Population and Traitement			
FGP	47.034*	238.325***	31.633**		
MTG	10.012^{ns}	25.044*	47.378*		
Population	Bou Salem	Menzel Bouzelfa			
FGP	52 (5)^{b}	58 (3)^{a}			
MTG	6.02 (0.45) ^{a}	5.51 (0.25)^{a}			
Traitement (mM)	0	50	100	150	200
FGP	86 (8)^{a}	77 (5)^{a}	52 (3)^{b}	41(4)^{c}	17 (3)^{d}
MTG	3.83 (0.22) ^{a}	4.43 (0.22)^{ab}	5 (0.54)^{b}	6.06 (0.59) ^{c}	9.52 (1.26)^{d}

F-probabilities are indicated by symbols: ns = non significant differences ; * significant differnces at $p < 0.05$. **significant differnces at $p < 0.01$. *** significant differences at $p < 0.001$. Values in parentheses are standard errors of the mean. For each column, values with the same letter indicate no-significant differences at 5%.

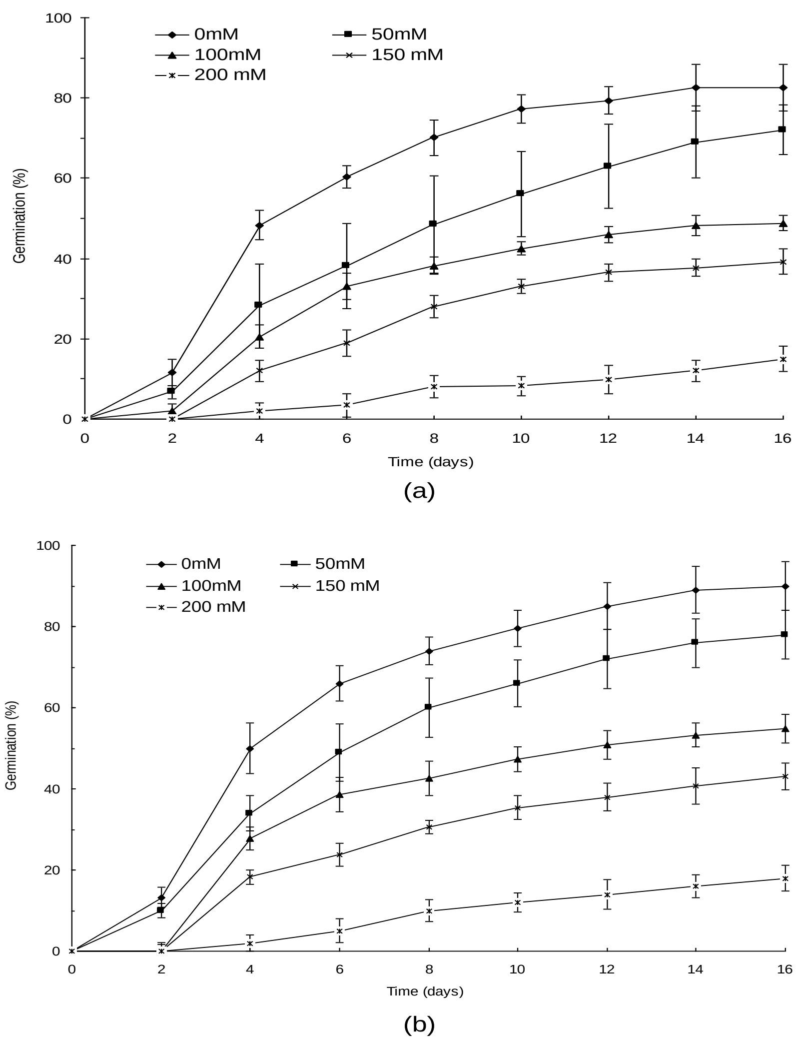


Figure 2. Cumulative germination percentage of two *C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa seeds incubated at different levels of salinity over a period of 16 days. The data are means values of four measurements. The confidence intervals were calculated at the threshold of 5%.

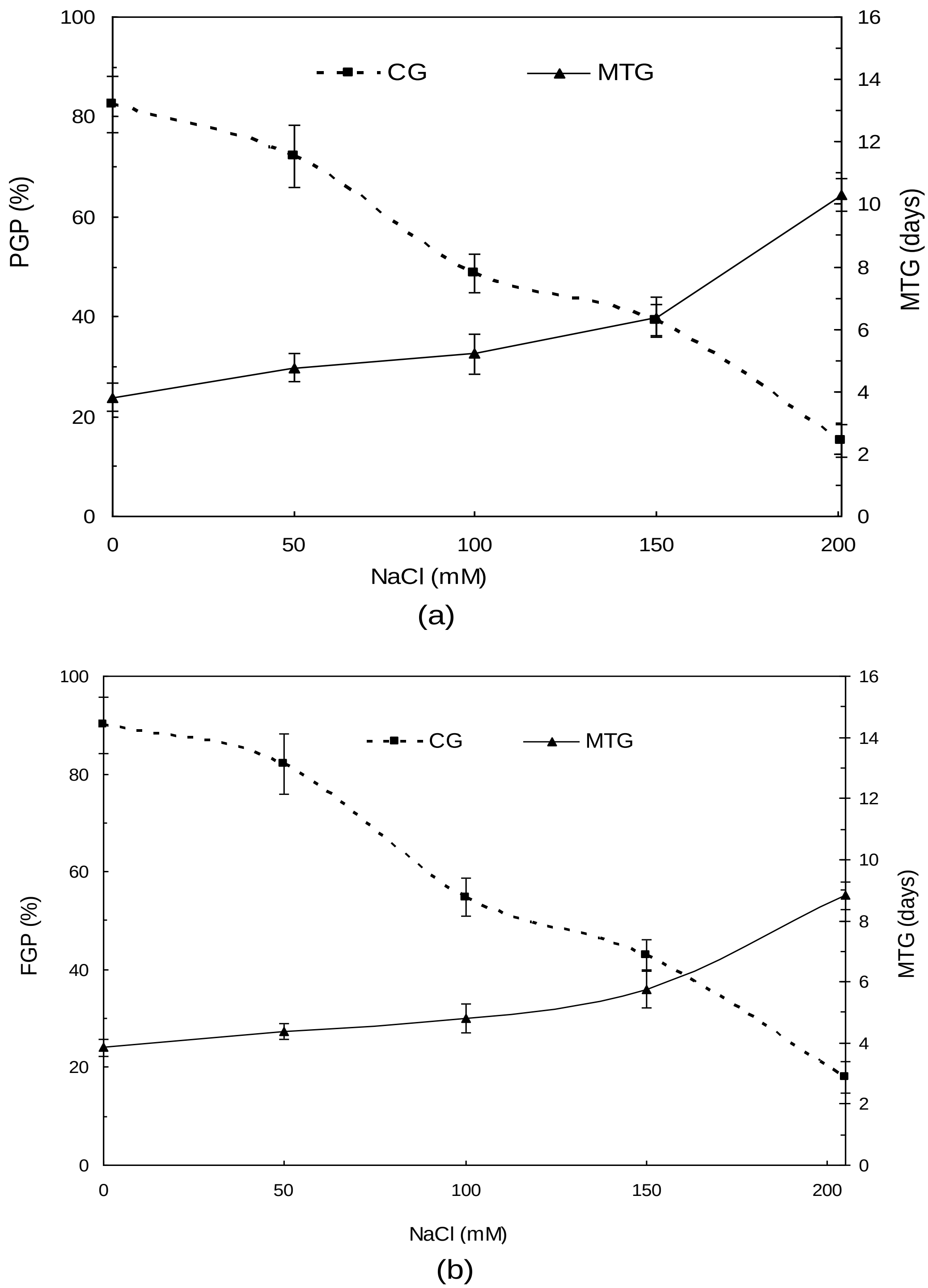


Figure 3. Effect of salinity on the mean germination percentage of two *C. C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa seeds incubated for 16 days at 30°C. The data are means values of four measurements. The confidence intervals were calculated at the threshold of 5%.

parameter reached 48% at 30°C. Up to 100 mM NaCl, germination started on day 4, whereas, at 150 and 200 mM, it witnessed a delay of 2 days. Maximum percentage in the two varieties was obtained on day 14. Under salt stress ´Menzel Bouzelfa´ variety had highest germination rate and ´Bou Salem´ had the least. At 200 mM, FGP decreased with 82 and 80% respectively ´Bou Salem´ and ´Menzel Bouzelfa´, compared with the control (Figure 3).

Polynomial regression analysis was used to determine

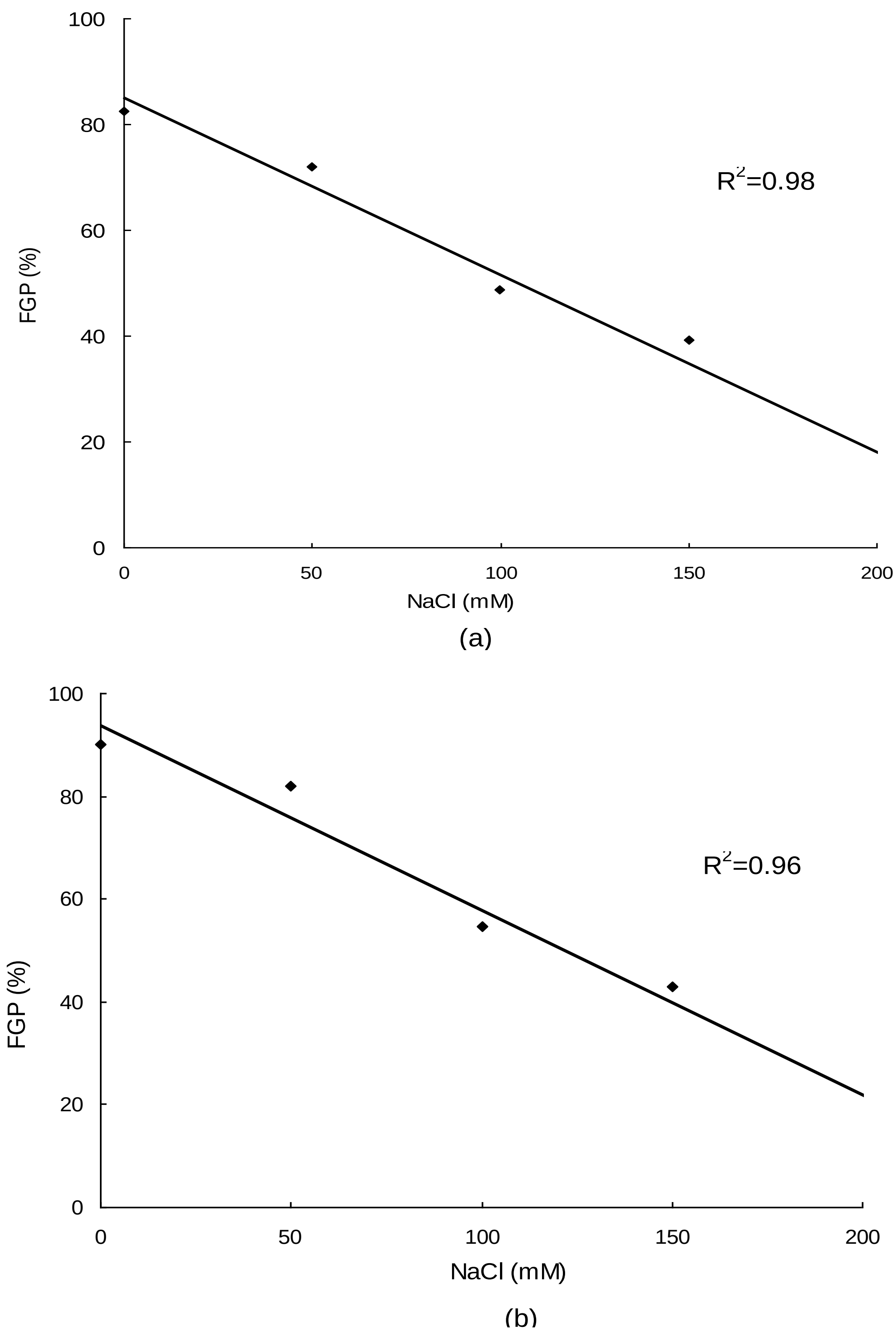


Figure 4. Regression plot for mean germination percentage of two *C. C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa seeds at different NaCl concentration at 30°C.

the relationships between germination percentages and salinity at optimal temperature (30°C). There was a strong negative relationship between germination and salinity with a regression coefficient of 0.95 (Figure 4). Nevertheless, a clear distinction between the effect of salt on the germination percentage and germination rate is evident when examining (Figure 5). Actually, salt stress affected significantly germination percentage, (estimated

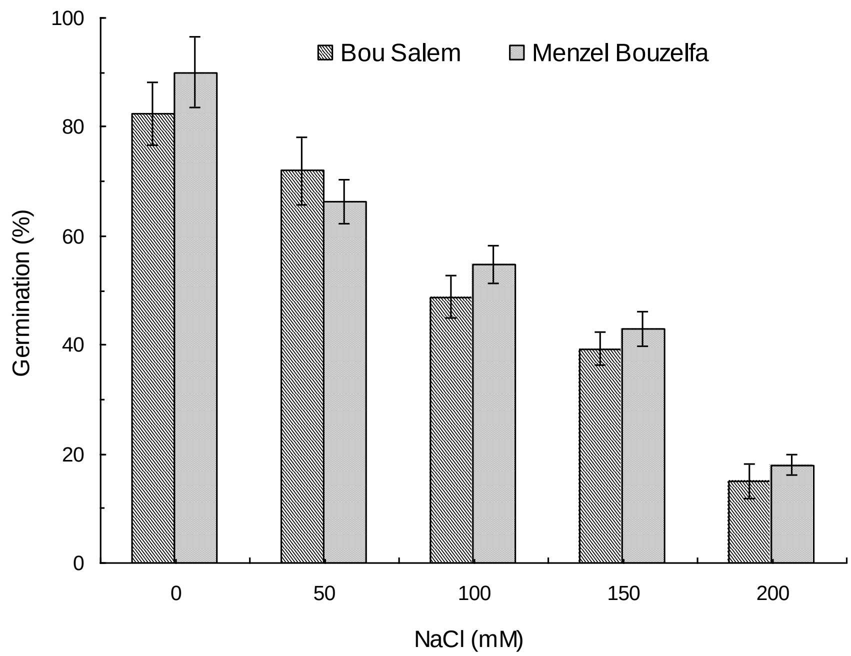


Figure 5. Effect of various NaCl concentrations on germination percentage and MTG of two *C. C. olitorius* varieties (a) Bou Salem and (b) Menzel Bouzelfa.

by the increase of MTG).

DISCUSSION

Little is known about seed germination seed germination of Tunisian leafy vegetable to abiotic stress. The studies were carried out to observe the influence of temperature and salinity on germination seeds of two varieties of *C. olitorus.* Substantial variation was observed in our study for traits related to the adaptation of *C.olitorus* to temperature and salt stress. The varieties for germination traits was mainly explained by geographical origin, suggesting that those traits are mainly constitutive and result from natural selection pressure exerted by the climatic constraints (Soltani et al., 2002). Although data were obtained from seeds germinated in Petri dishes, the result can be related to *in situ* performance. ´Menzel Bouzelfa´ variety, which is better adapted to naturally occurring salt stress, was more tolerant to experimentally imposed salt stress than ´Bou Salem´ variety (Table 1 and Figure 3).

Optimal germination percentage occurred at 30°C (Figure 1). Germination was inhibited by either an increase or a decrease in temperature from the optimum. This temperature corresponds to the ecological adaptation of natural habitats of *C. olitorus* where seeds reach maturity in summer (Nkomo and Kambizi, 2008). Several researches have reported the importance of temperature during seed development and maturation as an important factor affecting seed germination (Allen and Meyer, 2002). Ekstam et al. (1999) has elucidated the importance of fluctuating temperature as a requirement for germination and that in addition to being a sensor for ´exposed´ seed sites; it is also effective as a germination timing sensor. A slight increase or drop in temperature may affect a number of processes determining the germinability of seeds, including membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Bewley and Black, 1994). Shimono and Washitani (2004) suggest that the higher temperature range in the decreasing temperature regime induces secondary dormancy. Tolerance to salinity during germination could be decisive for the establishment of plants growing in saline soil (Ungar, 1995). Seed germination behavior in relation to thermal and salt stress is very important to determine the colonization capacity of the species (Ungar, 1982). Germination of the two *C. olitorus* varieties ´Bou Salem´ and ´Menzel Bouzelfa´ seeds was inhibited with an increase in salinity at the optimal temperature (30°C) (Table 1 and Figure 3) and little germination was observed at 150 and 200 mM NaCl. Similar results were reported for *Atriplex griffithii* (Khan and Rizvi, 1994), and *Cressa cretica* (Khan, 1991). *Cymbopogon schoenanthus* (Khadhri et al., 2010). Those results corroborate several other studies, showing that halophytes, as glycophytes, are especially sensitive to salt during the germination phase (Khan et al., 2002). In fact, Chartzoulakis and Klapaki (2000) demonstrated that 50 mM salinity in the external medium delayed germination in two pepper hybrids, but did not reduce the accumulated percentage of germination observed at the end of the experiment; whilst the ability to germinate became significantly reduced at 100 and 150 mM NaCl concentrations. Reduction germination rate under salinity conditions have been demonstrated by Murillo-Amador et al. (2002) and Soltani et al. (2006). Both osmotic and salt toxic effects have been implicated in germination inhibition (Machado et al., 2004). Foolad and Lin (1997) examined the germination of tomato seeds in different ionic and non-ionic germination mediums with identical osmotic potentials. They concluded that tomato germination rate was mainly affected by the osmotic effect of the medium, and just secondarily by its ionic effect. Our results reveal important reductions in germination rates in those seeds subjected to the highest NaCl concentrations 150 and 200 mM. This may indicate that seed osmotic adjustment was affected and that stress had possibly favoured the entering of other ions into the seeds. Smith and Comb (1991) attributed this to low humidity content, which may have increased saline stress, caused cessation of metabolism or inhibited certain stages in the germination metabolic sequence. Shokohifard et al. (1989) found two ways in which saline stress negatively affected radish seed germination: i) osmotically, by reducing water absorption and ii) ionically, by accumulating Na^+ and Cl^-, thereby altering nutrient uptake balance and causing a toxic effect.

Under such conditions, changes in the incubation temperature, particularly at high salt concentration, may result in malfunctioning of enzymatic systems. This situation would lead to limitations in many physiological processes vital to seed germination. The present study showed that seeds of two *C. olitorus* varieties germinated better in the intermediate incubation temperature (20, 25, 30, and 35°C). *C. olitorus* seeds experienced maximum germination at 30°C in darkness for all NaCl concentrations tested. Salt stress decreased both germination percentage and germination rate (increase of the MTG).

Conclusion

Results of this study demonstrate that salt tolerance during germination exists within *C. olitorus* varieties which represent a genetic material for development of salt tolerance of *C. olitorus*. Further investigations are necessary to understand the ecophysiological strategies of this species for the survival under natural environmental conditions.

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