



Full Length Article

Effect of Drought and Salt Stresses on Germination and Early Seedling Growth of Different Color-seeds of Sesame (*Sesamum indicum*)

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Abstract

High salinity and drought are common environmental problems affecting seed germination and plant growth especially in arid and semi-arid regions. Sesame (*Sesamum indicum* L.) is an oilseed crop rated moderately salt and drought tolerant but it is sensitive at germination and seedling stages. The effect of salt stress (NaCl) and water stress (PEG-6000), using the same osmotic potential (0 to -1.4 MPa), on germination and early seedling growth of sesame genotypes with different color seeds was studied. Germination percentage (GP), germination rate (GR), mean germination time (MGT), root length (RL) and shoot length (SL) were investigated. Drought and salt stresses, seed color and interaction of each stress with seed color had a significant effect on all studied parameters ($P < 0.01$). Yellow and brown seeds exhibited higher GP and GR and lower MGT than white and black seeds in absence and presence of both stresses. Germination was reduced and delayed by both stresses. However, it continued at all concentrations of NaCl, while it ceased at -1.0 MPa of PEG. Reduction in GP and GR was gradual for yellow and brown seeds and drastic for white and black ones, already from -0.2 MPa of PEG and NaCl. At equivalent osmotic potential, salt stress had a lower inhibitory effect on germination and seedling growth than drought stress. For Moroccan cultivars, having yellow and brown seeds, seedling growth was more affected by both stresses than seed germination, thus RL and SL could be taken as selection criteria for drought and salt tolerance at early growth stages. Sesame cultivars having intermediate seed colors (brown and yellow) could be recommended for moderate saline soils. © 2016 Friends Science Publishers

Keywords: Sesame; Seed color; Germination; Salinity stress; Drought; Seedling growth

Introduction

Drought and salinity are two major abiotic stresses due to their wide occurrence and high magnitude of their impact (Bartels and Sunkar, 2005). Severe drought and high salinity could promote land desertification and salinization, processes which are rapidly increasing on a global scale. More than 10% of arable land has become desertified or salinized, and average yields of major crops have been reduced by more than 50% (Wu *et al.*, 2011). Salinity and drought stresses are physiologically related because both induce osmotic stress and most of the metabolic responses of stressed plants are similar (Kumar *et al.*, 2011). Water stress effects on plant growth and seed yield are genotype-dependent (Bannayan *et al.*, 2008) and also depend on the timing, duration and magnitude of the water deficit (Pandey *et al.*, 2001). Salt stress adversely affects the growth of plants during all development stages (Jamil *et al.*, 2006). Seed germination is the beginning of plants life cycle and it is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri *et al.*,

2001). Germination and seedling growth declined with many abiotic factors such as salt and drought stresses which are two of the most important abiotic stresses that limit number of seedling and seedling growth (Almansouri *et al.*, 2001; Farooq *et al.*, 2009, 2015). Salinity has also been identified as the major factor influencing seedbed establishment in arid and semi-arid regions (Almansouri *et al.*, 2001). Increasing salinity can influence seed germination either by toxic effects of specific ions, such as Na, Cl and SO₄, or by creating osmotic pressure which impedes seed water uptake (Kaya *et al.*, 2006; Shaikh *et al.*, 2007). Soil salinity and water deficit decrease the soil water potential that leads to late and inadequate germination associated with failure of stand establishment. As a result, the crop productivity is adversely affected (Willenborg *et al.*, 2005).

Sesame (*Sesamum indicum* L.) is well known as one of the oldest oil crops used by humans for thousands of years for edible oil, paste, cake and confectionary purposes (Weiss, 2000). The chemical composition of sesame seed shows that it is an important source of oil (50–60%), protein

(18–25%), carbohydrate and ash (Elleuch *et al.*, 2007; El Khier *et al.*, 2008). Seed oil is highly unsaturated edible oil, rich in essential fatty acids such as linoleic acid. Sesame seed oil contains almost equal levels of oleic (35 to 54%) and linoleic (39 to 59%), 10% of palmitic acid and 5% of stearic acid (Hall, 2003). It is also rich in various bioactive compounds including phytosterols, tocopherols and lignans such as sesamin, sesamol and sesaminol, which are known to play an important role in providing stability against oxidation of oil and contribute to antioxidative activity (Al-Yemeni *et al.*, 2000).

Compared to other crops, sesame has better drought tolerance; however, it remains particularly sensitive to drought occurring during germination and seedling stages (Orruno and Morgan, 2007; Boureima *et al.*, 2011). In fact, germination and seedling growth are reduced under drought; however magnitude of decrease varied according to the drought level and the cultivar (Heikal *et al.*, 1982; Mensah *et al.*, 2006; Hassanzadeh *et al.*, 2009; Boureima *et al.*, 2011; Bahrami *et al.*, 2012). Sesame is often grown under irrigation conditions in arid and semi-arid areas, which are characterized by high temperatures, high levels of solar radiation, high evaporative demand and unpredictable occurrence of drought (Witcombe *et al.*, 2008; Hassanzadeh *et al.*, 2009). All these combined factors cause an increasing soil salinity which is a serious constraint limiting the production of this crop. However, just few studies have been carried out on the effect of salt stress on sesame seed germination. These showed that germination and early seedling growth parameters were negatively affected and the effect severity varied according to the salt stress level and the cultivar (Purohit *et al.*, 2005; Ramirez *et al.*, 2005; Bahrami and Razmjoo, 2012). No previous research achieved in regard to sesame germination under drought and salt stress conditions has considered the seed color effect. Furthermore, no comparative study on the effect of drought and salt stress on germination and early seedling growth parameters has been conducted. This study was, therefore, carried out to evaluate and compare the effect of salt stress (NaCl) with that of water stress (PEG) on sesame germination and early seedling growth, and various cultivars characterized by different seed colors.

Materials and Methods

The experiment was conducted in the laboratory of Bioprocess and BioInterfaces, Faculty of Science and Technology of Béni-Mellal, Morocco. The used plant material consisted of four sesame genotypes: Two Moroccan cultivars and two American accessions. Moroccan cultivars, characterized by yellow and brown seeds, were collected through a survey conducted in Tadla-Azilal region, the most important area of sesame cultivation in Morocco. American accessions, characterized by black and white seeds, were provided by Dr. Bradley Morris from ARS-USDA (Griffin, GA). Seeds of these materials were

increased and samples of the new obtained seeds were experimented in this study.

Drought stress was induced by polyethylene glycol (PEG-6000) treatment. Seven levels, with different osmotic potentials of -0.2, -0.4, -0.6, -0.8, -1, -1.2 and -1.4 MPa, were prepared and used as described by Michel and Kaufmann (1973). Salt stress, with the same osmotic potentials (from -0.2 to -1.4 MPa), was adjusted using NaCl, according to Coons *et al.* (1990). Distilled water served as a control.

The experiment was conducted in a double factorial completely randomized design, with three replications. The first factor was the seed color (genotype), with four levels, and the second was the stress solution (NaCl or PEG), with eight levels.

For each treatment, 100 seeds of each genotype cultivar were allowed to germinate in a Petri dish on filter paper moistened with 10 mL of appropriate levels of NaCl and PEG-6000. The Petri dishes were covered to prevent moisture loss by evaporation. The experiment was conducted at 25°C in an incubator for seven days and 2 mL of each solution was added to Petri dish every 24 h. When the radicle reached up 2 mm length, the seed was considered germinated (ISTA, 1985). Germinated seeds were counted at regular intervals every day until the end of the experiment period (seven days). Germination percentage (GP), determined on the seventh day, and germination rate (GR) were calculated as follows (Farooq *et al.*, 2005):

$$GP = (N7/100) \times 100$$

$$GR = \sum [(Gi - Gi-1)/i].$$

N7 = number of seed germinated on the 7th day

Gi = number of seed germinated on day i

Gi-1 = number of seed germinated on day i-1

Mean germination time (MGT), expressed in d, is the inverse of GR (MGT = 1/GR). Root length and shoot length, expressed in cm, were measured on the seventh day after end of experiment (Bray, 1963). Shoot length was measured from the cotyledons to the collar, and the root length was measured from the collar to the root tip.

Analysis of variance (ANOVA) to determine statistically significant differences among seed colors, salinity or drought levels and the interactions seed color by salinity and seed color by drought was performed. Duncan's new multiple range test was applied to compare treatment means. The used statistical program was SAS 9.1.3 for windows.

Results

Effects of Water and Salt Stresses and Seed Color on Seed Germination

Both water stress and salt stress had a significant effect on germination percentage (GP), germination rate (GR) and mean germination time (MGT) of all sesame seed types

(colors) ($P < 0.01$). However, inhibition of germination was more pronounced in case of water stress, using PEG (Fig. 1). There were significant differences between seed types for these parameters. In absence of any stress, GP was around 40% for black seed, 56% for white seed and 100% for yellow and brown seeds (Fig. 1), while GR was 19, 26, 99 and 98%, respectively (Fig. 2). The corresponding MGT was 0.054 d for black seed, 0.040 d for white seed and 0.010 d for yellow and brown seed (Fig. 3). There was a significant effect of seed color \times drought and seed color \times salinity interactions on those parameters ($P < 0.01$), indicating that the seed types reacted differently to the level of both stresses. Yellow and brown seeds continued to germinate until -1.4 MPa of NaCl and -1 MPa of PEG. There was a gradual reduction in GP with increase in the level of water and salt stress i.e., decreasing the osmotic potential from -0.6 to -1.2 MPa of NaCl solutions and from -0.4 to -1 MPa of PEG solutions (Fig. 1). Under salt stress, drastic reduction of GP (Fig. 1) and GR (Fig. 2) and the drastic delay of MGT (Fig. 3) occurred at -1.4 MPa, whilst under water stress, they occurred at -1.0 MPa. Below this osmotic potential, no germination was recorded (Fig. 1). For white and black seeds, there was a drastic decrease of GP (Fig. 1) and GR (Fig. 2) and a consequent delay of MGT (Fig. 3) already from -0.2 MPa of PEG and NaCl solutions, indicating the sensitivity of such seeds to low level of water and salt stresses. White seed stopped to germinate at or below -1 MPa of PEG and at -1.4 MPa of NaCl, while black seed stopped to germinate at or below -0.8 MPa of PEG and at -1.4 MPa of NaCl (Fig. 1). Thus, yellow or brown seeds of the Moroccan cultivars exhibited higher GP and GR and lower MGT than white and black seeds of American accessions, in absence of stress and under different levels of drought and salt stress. Besides, for all seed types, both stresses affected and delayed seed germination. However, at equivalent level of stress (same osmotic potential), NaCl had lower inhibitory effect than PEG.

Effects of Water and Salt Stresses and Seed Color on Early Seedling Growth

Drought and salt stress had a significant effect on root length (RL) and shoot length (SL) of all seed types ($P < 0.01$). However, for both root length (Fig. 4) and shoot length (Fig. 5), inhibition of elongation was more pronounced in case of water stress using PEG, compared to salt stress using NaCl. Also, there were significant differences between seed types (colors) for root and shoot length. In absence of any stress, RL was 5.50 cm for black seed, 5.44 cm for brown seed, 5.05 cm for yellow seed and 4.60 cm for white seed (Fig. 4), while SL was 5.57, 4.10, 4.60 and 5.02 cm, respectively (Fig. 5). Under drought stress (PEG) and salt stress (NaCl), RL and SL decreased for all seed types by increasing level of these stresses i.e., decreasing the osmotic potential. However, the decrease was, in general, greater for PEG than NaCl (Fig. 4 and 5).

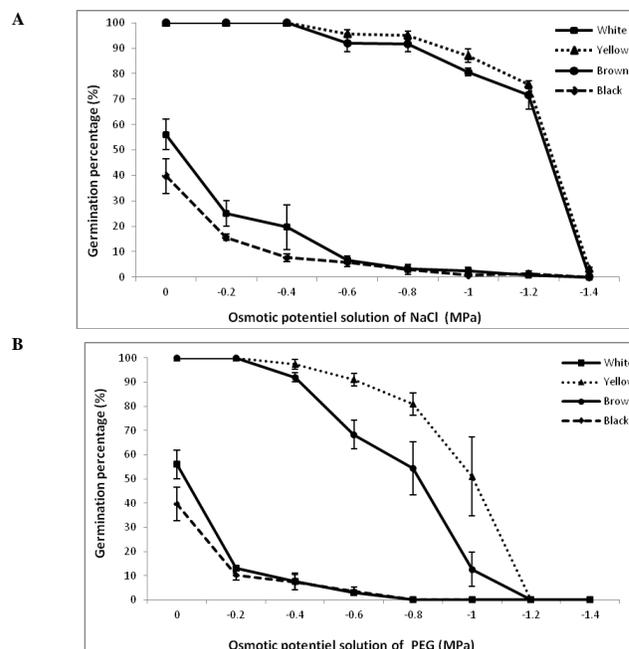


Fig. 1: Variation of germination percentage of four sesame genotypes with different color seeds germinated in different osmotic potential (OP) solutions of NaCl (A) and PEG (B). Vertical bars indicate \pm standard error

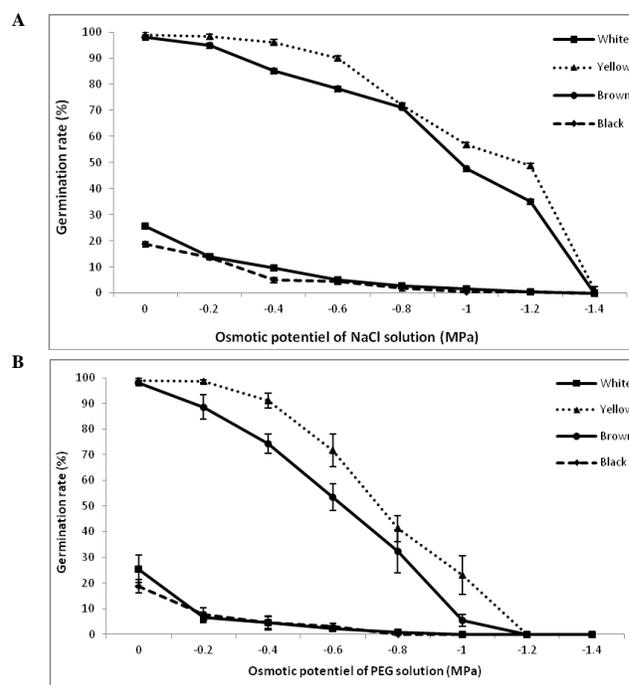


Fig. 2: Variation of germination rate of four sesame genotypes with different color seeds germinated in different osmotic potential (OP) solutions of NaCl (A) and PEG (B). Vertical bars indicate \pm standard error

At the same osmotic potential, and for all seed types, PEG had higher inhibitory effect on RL and SL than NaCl. Similarly to seed germination parameters, there was significant interaction seed color \times drought and seed color \times salinity on early seedling growth parameters ($P < 0.01$). For both RL and SL, seed types reacted differently to the level of both stresses. Under intermediate salt stress (-0.6 MPa of NaCl), RL was reduced by 58% for white seed, 40% for black seed, 22% for brown seed and 16% for yellow seed (Fig. 4), while SL was reduced by 54, 48, 32 and 59%, respectively (Fig. 5). In case of severe salt stress (-1.2 MPa of NaCl), RL was decreased, respectively, by 88, 86, 91 and 90% (Fig. 4), and SL was decreased, respectively, by 88, 87, 88 and 84% for these four seed types (Fig. 5). These results indicate that, for early seedling growth, yellow and brown seeds were globally the least affected by intermediate and severe salt stress. Under intermediate water stress (-0.4 MPa of PEG), RL was reduced by 58% for black seed, 55% for brown seed, 48% for white seed and 45% for yellow seed (Fig. 4), while SL was reduced by 46, 71, 38 and 64%, respectively (Fig. 5). This indicates that, surprisingly, early seedling growth of black and white seeds would be less affected than that of yellow and brown seeds under intermediate drought. Under relatively severe water stress (-0.8 MPa of PEG), there was no germination for black seeds (Fig. 1), while RL was decreased by 74, 88 and 96% for brown, yellow and white seeds, respectively (Fig. 4). SL was reduced by 81, 86 and 97%, respectively for these three seed types (Fig. 5). Thus, regarding early seedling growth, brown seed was the most tolerant to relatively severe drought, followed by yellow seed. White seed was the most sensitive.

Discussion

All the seed germination parameters measured, GP, GR and MGT, were affected by both water and salt stresses. GP and GR were decreased, while MGT increased with increase in stress levels. However, yellow and brown seeds of the local Moroccan cultivars were less affected than white and black seeds of the American accessions, indicating that Moroccan genotypes were more tolerant to both stresses than American ones. Also, regarding seedling growth parameters, RL and SL have been affected by water and salt stress. They decreased with increased stress levels. Nevertheless, brown and yellow seeds were less affected than white and black seeds for these parameters. Thus, Moroccan genotypes were more tolerant to both stresses than American ones. Previous studies on sesame had also evidenced the effect of genotype and drought level (Boureima *et al.*, 2011; Bahrami *et al.*, 2012; Keshavarzi, 2012) and the effect of genotype and salt stress level (Bahrami and Razmjoo, 2012; Tabatabaei and Naghibalghora, 2014) on seed germination and seedling growth parameters. Reduction in germination parameters may be attributed to lower infusibility of water through the

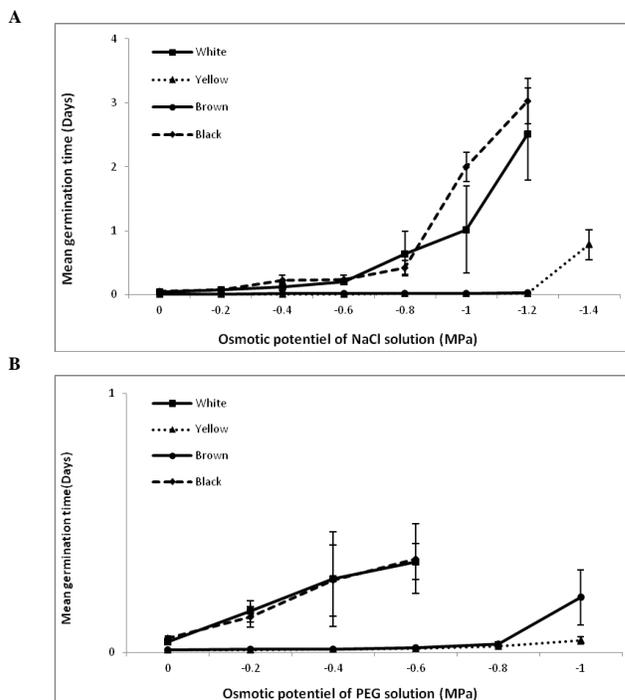


Fig. 3: Variation of mean germination time of four sesame genotypes with different color seeds germinated in different osmotic potential (OP) solutions of NaCl (A) and PEG (B). Vertical bars indicate \pm standard error

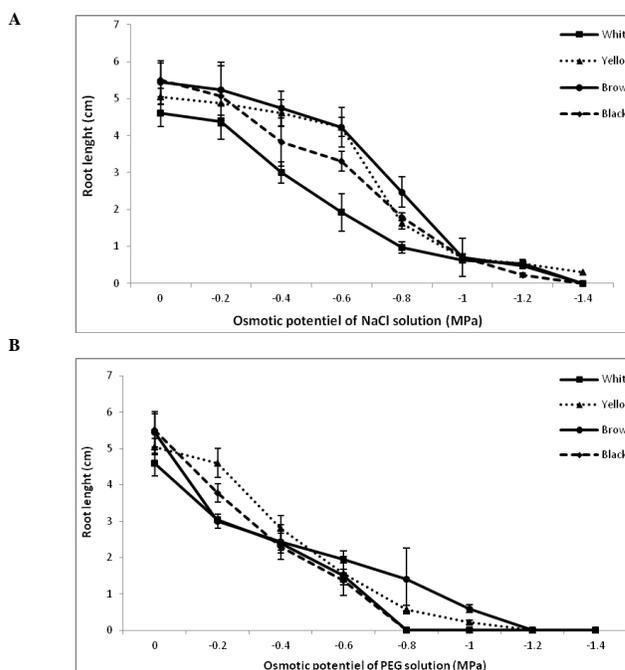


Fig. 4: Variation of root length of four sesame genotypes with different color seeds germinated in different osmotic potential (OP) solutions of NaCl (A) and PEG (B). Vertical bars indicate \pm standard error

seed coat and initial water uptake of the seed under stress condition (Khayatnezhad and Gholamin, 2011; Bahrami, *et al.*, 2012). Natural color of sesame seeds varies from black, intermediate colors (e.g., brown, golden, yellow) to white (Zhang *et al.*, 2013). Differences in the behavior of such seeds during germination can be observed. Because of color pigment in seed coat, seed color influences water uptake, gas diffusion, seed dormancy, seed quality, germination and seedling emergence in some crops (Atis *et al.*, 2011). Our findings are in agreement with those of Suma and Srimathi (2014) who had reported that sesame seeds with dark brown color recorded better germination percentage in comparison with black and creamed sesame seed due to their better stamina in physical, physiological and biochemical phenomenon. Also, Yao *et al.* (2010) concluded that brown seeds of *Chenopodium album* were salt tolerant and could germinate rapidly to a high percentage in a wide range of environments; while black seeds were salt sensitive, indicating that the seeds with different colors react differently to germination conditions. To germinate, yellow and brown seeds could tolerate till -1 MPa of PEG, and from -1.2 MPa, no germination was recorded. Similarly, for sunflower, none of the seeds could germinate at -1.2 MPa (Kaya *et al.*, 2006). However, in a recent study on safflower, it was shown that seeds ceased to germinate already from -0.25 MPa (Zraibi *et al.*, 2011), indicating that sesame is more tolerant than safflower to drought stress during germination stage. To germinate, yellow and brown seeds could tolerate till -1.2 MPa of NaCl. However, a drastic decline in germination features was observed from -1.2 to -1.4 MPa. This could be explained by the fact that the osmotic potential -1.2 MPa is the critical salinity tolerance level or threshold. It seems that at osmotic potential -1.4 MPa and below, water potential inside the germinating seed became higher than that outside, i.e. in NaCl substrate and consequently there is no more water uptake through the seed coat, which causes germination failure. Additionally, it is assumed that extreme salinity might be toxic to the seed embryo and thus germination is severely inhibited. This is in accordance with findings of Bahrami and Razmjoo (2012) who had reported, for some sesame cultivars, a sharp decline in germination, from 50–56% to 0–5%, when salinity level was increased, respectively from 14.65 to 18.45 dS m⁻¹. Also, drastic reduction was also observed in rapeseed germination, from 80% at -0.9 MPa to 0% at -1.2 MPa (Pace and Benincasa, 2010).

For all seed types (colors), NaCl and PEG adversely affected the germination and early seedling growth of sesame. This is may be due to alteration of enzymes and hormones found in the seed (Botia *et al.*, 1998) or to the metabolic disorders induced by stress and generation of Reactive Oxygen Species (Almas *et al.*, 2013). It could also be a deficit of hydration of the seeds due to high osmotic potential causing inhibition of the mechanisms leading to the output of the radicle out of the coat and therefore a seed germination delay (Gill *et al.*, 2001; 2003).

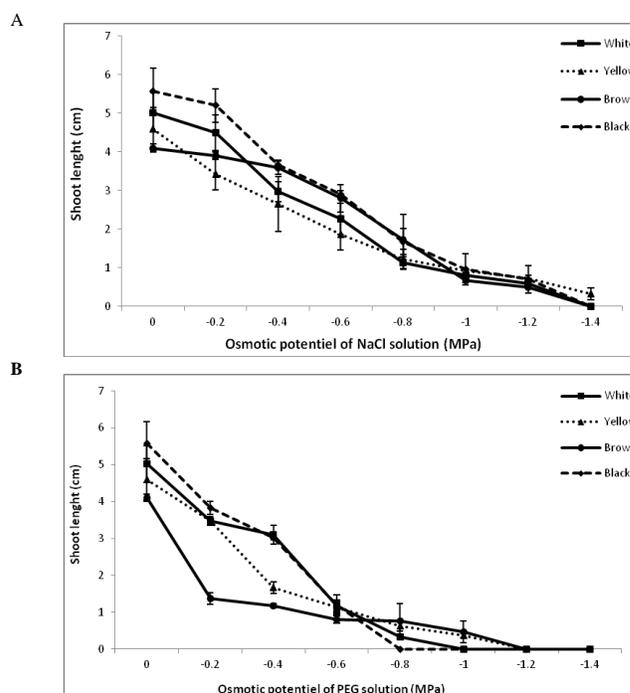


Fig. 5: Variation of shoot length of four sesame genotypes with different color seeds germinated in different osmotic potential (OP) solutions of NaCl (A) and PEG (B). Vertical bars indicate \pm standard error

However, higher germination percentage under NaCl stress compared to that of PEG, at the same water potential, indicated that adverse effect of PEG on the germination was due to osmotic effect rather than specific ion accumulation (Zhang *et al.*, 2010). These findings are in accordance with those of other studies on various species, such as barley (Zhang *et al.*, 2010), safflower (Zraibi *et al.*, 2011), sunflower (Kaya *et al.*, 2006), soybean (Khajeh-Hosseini *et al.*, 2003), durum wheat (Sayar *et al.*, 2010), pea (Okçu *et al.*, 2005) and cowpea (Murillo-Amador *et al.*, 2002). This demonstrates that for the same water potential, water stress is more severe than salt stress. For NaCl treated seeds, not considering extreme salt concentrations, ion (Na⁺ and/or Cl⁻) uptake makes seeds and seedlings osmotic potential more negative than that of external substrate. In other words, the accumulation of ion by the imbibing seed embryo leads to create a water potential gradient between the embryo and substrate, and thus maintain water uptake during seed germination (Dodd and Donovan, 1999; Zhang *et al.*, 2010; Shitole and Dhupal, 2012). PEG molecules do not enter in the seed and once water potential of the seed and its surrounding environment are in equilibrium, the seed will not continue to imbibe (Michel, 1983; Mehra *et al.*, 2003). Also, MGT in NaCl solution was lower than MGT in PEG solution for the same water potential. This could be explained, as mentioned above, by more rapid water uptake in NaCl

solutions. Similar findings were reported by Zhang *et al.* (2010) in barley, Khajeh-Hosseini *et al.* (2003) in soybean and Zraibi *et al.* (2011) in safflower.

On the other hand, by comparing the effect of PEG and NaCl on germination percentage and root and shoot length, water and salt stresses, regardless of their level, had lesser inhibitory effect on seed germination than seedling growth, for yellow and brown seeds characterizing the local Moroccan cultivars. This result agrees with that of Zraibi *et al.* (2011) having evaluated different safflower varieties under drought and salt stress. Contrarily, these stresses had less inhibitory effect on seedling growth than seed germination, for white and black seeds characterizing the American accessions. A similar finding was reported for sunflower (Kaya *et al.*, 2006). Thus, it could be suggested that, in sesame, early selection for drought and salt tolerance should be based on germination percentage for white and black seed types and on root and shoot length for yellow and brown seed types. In fact, it was reported that root and shoot length were the most important parameters in salt stress sensitivity evaluation, providing a relevant index to the response of plants to this stress (Jamil and Rha, 2004; Jamil *et al.*, 2006). This would indicate that, under high salinity environmental conditions, high root length and high shoot length at early seedling growth stage could be pertinent selection criteria for sesame salt tolerance breeding program.

Conclusion

Drought stress had more pronounced inhibitory effect on sesame seed germination and early seedling growth than salt stress. During germination and early seedling growth, Moroccan genotypes characterized by yellow and brown seeds were more tolerant to both stresses than American genotypes characterized by white and black seeds. This indicates that, contrarily to American cultivars, the Moroccan ones might be selected under drought and salt stresses conditions. Seedling growth was more affected by both stresses than seed germination for Moroccan cultivars, and thus root length and/or shoot length could be relevant selection criteria in sesame breeding program for drought and salt tolerance at early growth stages. Our findings may have agronomic and ecological implications, allowing intermediate seed colors (brown, yellow) sesame cultivars to grow in moderate saline soils. However, these cultivars should be further investigated at later growth stages to confirm their tolerance, evaluating the effects of both stresses on other morphological, physiological and agronomic attributes.

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