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Sensitivity of Seed Germination and Seedling Radicle Growth to Drought Stress in Sesame (*Sesamum indicum* L.)

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ABSTRACT

In order to study the effect of drought stress on germination indices in sesame (*Sesamum indicum* L.), a factorial experiment, using a completely randomized design with three replications was conducted. In this experiment, 22 confirmed sesame mutants induced through gamma ray treatment and their respective three parental sources were evaluated for five levels of drought stress (ψ_s= 0, -0.5, -1, -1.5 and -2 MPa) using aqueous solutions of PEG-6000. For each treatment, germination was checked every 48 h. Emerged seedling percentages as well as seedling root length were recorded after 7 days of experiment. Results evidence significant differences among genotypes and drought stress levels. Germination, emergence and root length were significantly reduced against control from a drought stress level ≤-1 MPa. Seeds did not germinate at all at an osmotic potential ≤-1.5 MPa whereas emergence was completely inhibited by -1 MPa. In present experimental conditions, a mild drought stress (-0.5 MPa) improved root growth in most genotypes studied as compared to controls. Emergence was the parameter the most affected by drought stress and germination the least affected. Genotypes hsc105, lc162, mc112, ef147 and hc107 were found to be more drought tolerant than their respective parents. Although sesame is reputed to be a drought tolerant crop, it is very susceptible to drought at germination stage and sufficient soil moisture is required for sesame seed emergence.

Key words: Ecophysiology, osmotic potential, germination, radicle length

INTRODUCTION

Sesame (*Sesamum indicum* L.) is often described as the oldest oilseed plant used by humans (Weiss, 2000) and was considered a drought-resistant crop (Fazeli *et al.*, 2006). The plant was initially domesticated on the Indian subcontinent (Bedigian, 2003). Plants in genus *Sesamum* produce unique chemical constituents not found in other edible oils that enable sesame oil to resist oxidative rancidity and thus contribute to its reputation of high quality oil, earning sesame the label "Queen of oilseed crops" (Al-Yemeni *et al.*, 2000; Bedigian, 2000). Sesame seeds contain 50% of oil and in a context of high demand for edible oil and biofuel, the latter could be an alternative source of income for small scale farmers allowing fighting against rural poverty.

One of the most important constraints for agriculture is water limitation (Xoconostle-Cazares *et al.*, 2010). The arid and semi-arid tropics where sesame is grown are characterized by high temperatures, high levels of solar radiation, high evaporative demand and unpredictable occurrence of drought (Witcombe *et al.*, 2007; Hassanzadeh *et al.*, 2009).

In the Sahel region, the raising and establishment phase of crops are generally marked by insufficient and irregular rainfall, separated by a number of drought-spells which can seriously affect crop development (El-Madidi *et al.*, 2005). In this context, a good germination capacity and seedling growth in water deficit conditions constitute interesting drought tolerance indices since they will allow predicting correct crop establishment.

Recent years, researchers have often used osmotica to lower the water potential (ψ_w) of plant growth media. This approach has many advantages: ψ_w can be controlled precisely whereas a large number of treatments can be performed quickly in a reproducibly way (Verslues *et al.*, 2006). Whereas osmotica with low molecular weight like mannitol freely penetrate the cell wall pores and cause plasmolysis rather than cytorrhysis, polyethylene glycol of molecular weight 6000 (PEG-6000) or above cannot enter plant cells pores (Ranjbarfordoei *et al.*, 2000). Because PEG-6000 does not enter the apoplast, water is withdrawn from the cell and the cell wall (Van den Berg and Zeng, 2006) without damaging cell content. We therefore, used PEG-6000 solutions which can mimic dry soil conditions more closely than solutions using low molecular weight osmotica which can be taken up by plant cells and can be toxic for plant growth. Almansouri *et al.* (2001) reported a delay and an inhibitory effect of PEG-6000 on germination of durum wheat (*Triticum durum* Desf.) seeds. Also, Maraghni *et al.* (2010) stated that osmotic stress induced by PEG-6000 significantly reduced seed germination in *Ziziphus lotus* whereas relative water content, length, height and dry weight of stem decreased with increasing osmotic stress level in *Lens culinaris* (Salehpour *et al.*, 2009).

The objectives of the present study were (1) to determine the level of drought stress that inhibits sesame seed germination and (2) to compare 22 gamma ray-induced mutant genotypes with their respective parents based on a number of drought tolerance indices trying to find some drought tolerant elite mutants at germination stage.

MATERIALS AND METHODS

Plant material development: The material of the present study consisted of 24 sesame lines (Table 1). These lines included 21 gamma-ray induced confirmed mutants and their three respective parental sources (32-15, 38-1-7, Birkan). Mutants were induced in 2008 and confirmed in 2009 (Boureima *et al.*, 2009; Diouf *et al.*, 2010). Two irradiation doses (300 and 400 Gy) were used for the three parents. The irradiated seeds (50 g dose^{-1}) were planted in the field and M2 seeds were harvested from these M1 plants. M1 plant progenies were grown in 30 m rows, 60 cm apart with plants at 20 cm within rows. After each ten rows, one parent control row (untreated) was sown. Potential mutants selected in M2 were grown as M3 generation to confirm true breeding behaviour during 2009 rainy season in progeny rows of 2 m long and 60 cm apart. These 21 mutant lines were selected based on various agronomic characters (e.g., seed colour, 5 plant yield, harvest index and precocity, plant architecture and closed capsules) for advanced screening.

Drought stress treatments: The experiment was conducted in factorial form, using a completely randomized design with three replications. Drought stress was induced by polyethylene glycol, PEG-6000 (M.W.6000, Across Organics, Geel) treatments. A range of water potentials

Table 1: Mutants, their main traits and respective parents

Mutant genotypes	Parent source	Main characteristics
ef 147	32-15	Early flowering
hb 168	32-15	High branching
icn 130	32-15	Multi capsules/leaf axil
lc 162	32-15	Capsules size and shape
mc 112	32-15	Multi carpel
hc 107	32-15	Hairy capsules
mt 169	32-15	Uniculm
vgr 156	32-15	Vigourous
cc 102	32-15	Closed capsules
ef 153	38-1-7	Early flowering
icn 141	38-1-7	Multi capsules/leaf axil
lc 164	38-1-7	Big capsules size and shape
mc 114	38-1-7	Multi carpel
hc 108	38-1-7	Hairy capsules
shi 165	38-1-7	Short internodes, uniculm
bc 167	38-1-7	Big capsules size and shape
ef 146	Birkan	Early flowering
icn 115	Birkan	Multi capsules/leaf axil
hsc 105	Birkan	Hairy stem and capsules
cc 103	Birkan	Closed capsules
wht 171	38-1-7	Whitish seeds, selected in M3
dwf 172	38-1-7	Dwarf (selected in M3)
32-15	Parent	Semi-branched, earliness
Birkan	Parent	Earliness, drought tolerant
38-1-7	Parent	Semi-branched

(0, -0.5, -1, -1.5 and -2 MPa) was produced via aqueous solutions of PEG-6000 according to Michel and Kaufmann (1973). Drought stress levels used were chosen with respect to the soil water potential conditions of the soils where these genotypes are usually grown. Seeds of each genotype were surface-sterilized by a 5 min treatment with a 6% calcium hypochlorite ($CaClO_3$) solution and then rinsed several times with distilled water. Thirty seeds per replicate and per treatment were allowed to germinate at 30±0.1°C in an incubator (BIN-90100214, Binder, Germany) on a sheet of Watman filter paper placed in Petri dishes for each experimental treatment. Five milliliter of PEG-6000 solution was added to Petri dishes every 48 h. In the case of the control treatment (0 MPa), 5 mL of distilled water was added. Petri dishes were covered to prevent loss of water by evaporation. The experiment was performed in the dark to allow root growth. For each treatment, germination was checked every 48 h and emerged seedling number as well as radicle length recorded after 7 days of experiment. Seeds were considered to have germinated when the radicle protruded from the seed coat (=1 mm) whereas emergence coincides with hypocotyl appearance. Radicle length was measured from 10 seedlings in each Petri dish using a digital caliper (Titan 23175, ±0.02 mm). Percentage of Germination (PG) and Percentage of Emergence (PE) for each genotype and treatment were then calculated. Genotype drought resistance was calculated as: Reduction rate (%) = (1- (Vs/Vp))×100 with Vs and Vp mean parameter performance value (such as number of germinated seeds or emerged seedlings, length of radical, under stress and non-stress conditions, respectively).

Data analysis: All statistical analyses were performed with Stata (v.11). ANOVA results were considered significant at $p<0.05$ and means comparisons were done with Tukey HSD test.

RESULTS AND DISCUSSION

Germination capacity: Mean germination percentage for all genotypes was 96% for control (0 MPa) and 76.35% at an osmotic potential of -1 MPa. Genotype hb168 had the highest germination percentage whereas the lowest percentage was recorded for cc103 (statistically similar to cc102, Table 2). The latter two genotypes (cc103 and cc102) are closed capsule mutants induced by large DNA deletions following gamma irradiation (Diouf *et al*., 2010). These mutations might affect some functions pleiotropically causing lower germination capacity. No germination at all was observed at an osmotic potential ≤-1.5 MPa. Similar findings were reported by Murillo-Amador *et al*. (2002) in cowpea (*Vigna unguiculata*). Tobe *et al*. (2006) reported that germination of three deciduous semi-shrubs of genus *Artemisia* was inhibited severely in PEG-6000 solutions at -1.2 MPa whereas *Ziziphus lotus* seeds germinated to 95 and less than 5% in PEG-6000 solutions of -0.4 and -1 MPa, respectively (Maraghni *et al*., 2010). Soil moisture is a crucial factor determining germination and seedling growth and therefore, plays an important role in determining the distribution patterns of species (Gutterman, 1993). Daws *et al*. (2008)

Table 2: Means values and means comparison per genotype for reduction percentage of germination (RPG), emergence (RPE) and radicle length (RPL)

Genotype	Germination (%)	Emergence (%)	Radicle length (mm)	RPG	RPE	RPL
32-15	90.74^{abc}	64.68^{a}	61.08^{a}	13.89^{abc}	52.98^{abc}	26.37^{abc}
mc 114	82.96^{c}	68.29^{a}	55.94^{a}	24.10^{abc}	47.57^{abc}	31.28^{abc}
wht 171	94.07^{abc}	64.43^{a}	52.60^{ab}	2.34^{c}	53.35^{abc}	43.51^{abc}
hc 108	93.33^{abc}	68.20^{a}	59.42^{a}	8.45^{abc}	47.70^{abc}	25.34^{abc}
dwf 172	87.41^{abc}	61.19^{ab}	55.26^{a}	16.00^{abc}	55.87^{abc}	22.55^{abc}
lc 164	96.67^{abc}	66.68^{a}	55.66^{a}	3.31^{c}	49.98^{abc}	29.67^{abc}
shi 165	95.19^{abc}	68.38^{a}	59.60^{a}	3.93^{c}	47.43^{abc}	37.58^{abc}
ef 153	97.78^{ab}	65.63^{a}	60.99^{a}	3.33^{c}	51.56^{abc}	35.45^{abc}
bc 167	96.30^{abc}	67.10^{a}	53.03^{ab}	3.93^{c}	49.34^{abc}	30.34^{abc}
icn 141	97.04^{abc}	66.90^{a}	62.92^{a}	4.44^{c}	49.65^{abc}	25.56^{abc}
38-1-7	98.52^{ab}	68.61^{a}	59.12^{a}	2.22^{c}	47.08^{abc}	21.68^{abc}
icn 115	88.89^{abc}	68.07^{a}	58.21^{a}	16.67^{abc}	45.49^{abc}	14.01^{abc}
Birkan	96.67^{abc}	71.32^{a}	58.00^{a}	5.00^{c}	43.02^{abc}	2.02^{c}
ef 146	88.89^{abc}	72.90^{a}	61.59^{a}	15.13^{bc}	40.65^{c}	18.48^{abc}
cc 103	47.41^{d}	45.87^{c}	26.96^{c}	35.10^{b}	73.85^{a}	57.12^{a}
hb 168	99.26^{a}	65.93^{a}	51.02^{ab}	1.11^{c}	51.11^{abc}	32.18^{abc}
mc 112	95.19^{abc}	65.56^{a}	62.46^{a}	5.59^{c}	51.67^{abc}	16.72^{abc}
ef 147	93.33^{abc}	64.85^{a}	65.64^{a}	4.96^{c}	52.73^{abc}	20.81^{abc}
hsc 105	84.44^{abc}	66.33^{a}	71.63^{a}	16.10^{abc}	48.04^{abc}	1.32^{c}
mt 169	86.30^{abc}	62.86^{ab}	53.39^{a}	19.35^{abc}	55.71^{abc}	37.59^{abc}
icn 30	87.04^{abc}	64.07^{a}	62.94^{a}	17.97^{abc}	53.89^{abc}	32.55^{abc}
vgr 156	95.93^{abc}	64.86^{a}	63.45^{a}	4.48^{c}	52.71^{abc}	29.39^{abc}
lc 162	97.04^{abc}	66.01^{a}	70.80^{a}	4.44^{c}	50.99^{abc}	7.46^{c}
hc 107	95.19^{abc}	66.83^{a}	65.07^{a}	5.54^{c}	49.76^{abc}	26.27^{abc}
cc 102	47.78^{d}	51.10^{abc}	32.25^{abc}	69.23^{a}	63.73^{ab}	54.16^{ab}

In each column, means with same letters are not statistically different at p = 0.05 level and Gorai *et al.* (2009) found that both seed germination rate and final germination percentage decreased with reduced soil water potential. Germination inhibition under low water potential might evidence an important survival mechanism that ensures dormancy in seeds of many crops within the soil seed bed until sufficient moisture is available for optimal seed germination and seedling establishment (Van den Berg and Zeng, 2006).

Many scientists reported seed germination at an osmotic potential below -1.5 MPa in numerous crops, including *Pennisetum glaucum* (L.) R. Br (Radhouane, 2008), *Sorghum bicolor* (Saint-Clair, 1976) and *Zea mays* (Verslues *et al.*, 1998). Seed germination assays can provide a quick test of salinity and drought response but must be interpreted with caution. The plant hormone abscisic acid (ABA) which is known to play a critical role in drought stress responses can interact with seed germination (Walton, 1980; Wang *et al.*, 2003; Angoshtari *et al.*, 2009). Lee *et al.* (2004) showed that exogenous ABA lowers germination in *Arabidopsis thaliana*.

The lowest Reduction Percentage of Germination (RPG) was recorded in hb 168 (1,11%) which was statistically similar with values recorded for genotypes 38-1-7, wht 171, lc 164, ef 153, bc 167, shi 165, icn 141, lc 162, vgr 156, ef 147, Birkan, hc 107 and mc 112 while the highest value was recorded in cc 102 (69.23%) (Table 3).

In present study, sesame seed germination was reduced by 22.43% at -1 MPa when compared to control (Table 4) and inhibited for all genotypes at an osmotic potential ≤-1.5 MPa which is close to wilting point in most sesame field conditions.

Emergence: The different drought stress levels tested had significant effect on seedling emergence. Emergence varied from 98.94% for controls to 5.62% at an osmotic potential of -1 MPa (Table 3). For the emergence reduction percentage, genotype ef 146 had the lowest reduction percentage (40.65%) whereas genotype cc 103 had the highest reduction emergence percentage (73.85%). At an osmotic potential of -1 MPa, emergence was reduced by a proportion of 94.26% and totally inhibited from -1.5 MPa downwards (Table 4) which is close to water potential at wilting point. In our conditions, emergence was the variable most affected by drought stress.

Table 3: Means comparison for percentages of germination, emergence and radicle length

Osmotic potential (Mpa)	Germination (%)	Emergence (%)	Radicle length (mm)
0	96.93±7.82[a]	98.94±3.11[a]	70.45±12.47[b]
-0.5	94.71±12.02[a]	90.63±15.53[b]	84.29±23.13[a]
-1	76.36±26.37[b]	5.62±9.24[c]	17.94±11.43[c]
-1.5	0[c]	0[d]	0[d]
-2	0[c]	0[d]	0[d]

In each column, means with same letters are not statistically different at p = 0.05 level

Table 4: Means comparison of germination, emergence and root length reductions caused by PEG-6000 treatments

PEG-6000 (MPa)	RPG	RPE	RPL
-0.5	2.09[c]	8.61[c]	-19.94[c]
-1.0	22.43[b]	94.26[b]	74.29[b]
-1.5	100[a]	100[a]	100[a]
-2.0	100[a]	100[a]	100[a]

In each column, means with same letters are not statistically different at p = 0.05 level, RPG: Reduction percentage of germination; RPE: Reduction percentage of emergence; RPL: Reduction percentage of radicle length

Radicle length: Radicle length was measured as a seedling vigour index. Two-way analysis of variance for radical length showed highly significant differences between the various drought stress levels and between genotypes ($p<0.01$) whereas the interaction was also highly significant. Radicle growth at lower osmotic potentials (higher PEG concentrations) was weak. In fact, a threshold level of hydration is required for radicle elongation to start (Ramagopal, 1990). It can be hypothesized that the presence of PEG drastically compromised water uptake. Mean average root length was 70.45 mm for controls and 17.93 mm at an osmotic potential of -1 MPa after 7 days of treatment. However, results show that radicle lengths were longer under osmotic potential of -0.5 MPa than under control conditions. Root lengths were increased by 19.94% at -0.5 MPa in comparison to controls while at low osmotic potential (-1 MPa) radicle lengths were reduced by 74.29% (Table 4). Similar results have been reported by Radhouane (2008) who stated that moderate drought stress increased root lengths of pearl millet cultivars by 15.8% whereas severe water deficit reduced root lengths by 88%. This mechanism was also reported in *Brassica oleracea* var.italica by Hegarty and Ross (1978). For the reduction percentage of radicle length (RPL), genotypes hsc 105, Birkan and lc 162 were shown to be the most tolerant genotypes whereas genotype cc 103, one of the closed capsule mutants, was the least tolerant. This reduction in radicle length following an exposure to drought stress could be due to a stop in division and cellular elongation at root level, leading to a kind of tuberization) and allowing seedlings to survive until conditions become favorable again (Frazer *et al*., 1990).

CONCLUSION

From present results, it can be concluded that in our experimental conditions, germination of sesame seeds was reduced by only 22.43% under an osmotic potential of -1 MPa and totally inhibited at an osmotic potential $\leq$-1.5 MPa which is close to wilting point in field conditions. A similar trend was observed in seedling emergence values which were however, more affected by low water potentials. Emergence was reduced by 94.26% at -1 MPa. Mild drought stress (-0.5 MPa) increased radicle length of sesame. In contrast, water potentials $\leq$-0.5 MPa significantly reduced root length of sesame. It appears that in insufficient soil moisture conditions, there are risks for sesame seeds to emerge. Thus, sufficient level of soil moisture is required for emergence and seedling establishment. Some mutants were found to be more drought resistant than their respective parents at germination stage. Finally, we succeeded to obtain drought tolerant lines at germination stage by using induced mutation technology.

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