Journal of Plant Biology, August 2007, 50(4) : 447-454

Germination Kinetics and Seed Reserve Mobilization in Two Flax (*Linum usitatissimum* L.) Cultivars under Moderate Salt Stress

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Because of its high contents of protein, α-linolenic-rich oil, lignans, and fiber, demand is increasing for flax (*Linum usitatissimum* L.) and flax seed oil as a food source. In this comparative survey, we examined germination and the mobilization of seed storage products (lipids and soluble proteins) of 3-d-old seedlings from two flax cultivars (N 51 and H 52) challenged with moderate salinity (up to 200 mM NaCl). At the highest salt concentration, germination appeared to be cultivar-dependent, with that of 'N 51' being less impaired and delayed than in 'H 52'. Sodium chloride inhibited germination via osmotic and toxic effects, so that seed viability was altered, especially in 'H 52'. At 200 mM NaCl, lipid mobilization was delayed in the earliest germination phases. This response was associated with increased proportions of linolenic acid contents in both cultivars and more linolenic acid-rich molecular species of TAGs. Irrespective of the salt level, soluble protein contents in both cultivars decreased over time, although a salt-related precocity of protein degradation occurred at 200 mM NaCl.

Keywords: flax, germination, lipids, proteins, salinity

Flax (*Linum usitatissimum* L., Linaceae) is a multi-purpose crop with benefits extending to both human and animal nutrition. This reflects its very high content of essential fatty acids (EFAs), a high percentage of dietary fiber, and the highest level of "lignans" from any plant or seed products used for human food (Oomah et al., 2006). Lignans are anti-carcinogenic compounds (Lay and Dybing, 1989). EFAs, mainly omega-6 (linoleic) and omega-3 (linolenic) fatty acids, are important in several metabolic processes, and serve as the structural components of cell membranes, as well as in energy transport, oxygen transfer, and hemoglobin production. For instance, α-linolenic acid (ALA) is the precursor of two other long-chain omega-3 fatty acids -- eicosapentaenoic acid (EPA) and docosahexaeonoic acid (DHA) – that play roles in preventing and reducing cardiovascular diseases (Bourlaye et al., 2004). Interest is growing in the medicinal benefits from flax seeds, particularly with respect to coronary heart disease, some kinds of cancer, and neurological and hormonal disorders (Huang and Ziboh, 2001; Simopoulos, 2002). Flax seeds, containing about 40% oil, have also long been used in industry as components of various paints or polymers (£ukaszewicz et al., 2004).

Germination is generally considered to be the developmental stage that is most salt-sensitive, especially for crops exposed to hostile environments (Ashraf and Wahid, 2000). The main salt-induced physiological disorder is diminished seed imbibition because of the low solute potential within the saline growth medium (Debez et al., 2004). This results in metabolic changes, including altered enzyme activities (Filho and Sodek, 1988), disturbances in N metabolism (Yupsanis et al., 1994), an imbalance in the levels of plant growth regulators (Ungar, 1978), and a general reduction in the hydrolysis and utilization of food reserves (Mondal et al., 1988; Ahmad and Bano, 1992). Salt stress may also impair germination due to its ionic effect. However, the relative importance of that factor is debatable because the phenomenon differs among species and even among cultivars of a same species (Dodd and Donovan, 1999).

Oilseed germination is characterized by the mobilization of storage lipids as a carbon source for the new seedling. Yet, those underlying mechanisms are only partially understood. The germination of lipid-rich seeds, such as rapeseed, involves, among other processes, the rapid transport of storage triacylglycerols (TAGs) in the cotyledons. Such hydrolysis of TAGs is catalyzed by highly active lipases (Ben Miled et al., 2000). This is the first step in TAG conversion to the sugars required for growth of the germinating embryo. Although the impact of salinity on storage-lipid degradation of oilseeds has been well documented (Younis et al., 1987; Ashraf and Wahid, 2000; Smaoui and Cherif, 2000), data for the flax response to this environmental issue are scarce, especially concerning germination and early seedling growth.

Here, we conducted a comparative survey of the germination kinetics and changes in lipid and protein contents in the seeds of two flax cultivars that were challenged with moderate salinity (100 to 200 mM NaCl).

MATERIALS AND METHODS

Germination

Seeds of two flax (*L. usitatissimum* L.) cultivars, 'H 52' and 'N 51', were supplied by the "Institut National de la Recherche Agronomique de Tunis" (INRAT, Tunisia). Twenty

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seeds each were germinated on sterile filter paper in Petri dishes that contained 5 mL of 0, 100, or 200 mM NaCl. The dishes (3 replicates per treatment) were incubated for 3 d in the dark at room temperature (20 ± 2°C). Germination was defined by the appearance of the emerging radicle. At regular intervals, the cotyledons were separated from the seedlings, rinsed with distilled water, and used for biochemical analysis. After 3 d, root lengths (cm) were measured and the non-germinated seeds were transferred to distilled water for a further 7 d to assess their viability. A theoretical model was used for more accurate monitoring of germination kinetics. Such a mathematical simulation, previously reported by Debez et al. (2004) for the oilseed halophyte *Cakile maritima*, hypothesizes that germination has a latent period of duration t_0, during which the seeds acquire the aptitude to germinate, followed by germination itself. After that latency, the probability k of germination per unit time is equal and constant with time for all seeds. This model is formulated as:

$$y(t) = y_{max} (1 + e^{-k(t - t_0)}) \quad (1)$$

Where y(t) represents the percentage of sown seeds that germinate at time t and y_{max} is the plateau (%) reached by y(t). The time (in days) for germination of 50% of the viable seeds is calculated as:

$$T_{50\%} = t_0 + \ln(2)/k \quad (2)$$

Lipid Analysis

Cotyledon total lipids were extracted according to the method of Allen and Good (1971). After the samples were fixed in boiling water for 5 min to inactivate their phospholipases, they were finely grounded. Lipids were extracted using a 50:50 mixture (v:v) of chloroform and methanol. These extracts were centrifuged at 3000 rpm for 20 min. The final chloroform extracts were evaporated with a rotary evaporator at 35°C, dried under a nitrogen stream, and stored at -10°C. Triacylglycerols (TAGs) were analyzed by HPLC on a Shimadzu apparatus equipped with an RP18 stainless steel column (250 mm long x 4.6 mm i.d.), a guard column, and a refractive index detector (differential refractometer). The mobile phase was acetonitrile:acetone (50:50, v:v), and the flow rate was isocratically controlled at 1.5 mL min^{-1}. TAG identifications were performed as reported by Gigliotti et al. (1993). A 10-µL sample was injected at each run. Fatty acids were methylated according to the technique of Metcalfe et al. (1966), as modified by Lechevallier (1966). Methyl esters were analyzed by GC, using an HP 4890 gas chromatograph equipped with a flame ioniation detector on a capillary column coated with supelcowax™ 10 (30 m long x 0.25 i.d., and 0.2 mm film thickness). Temperatures of the column, detector, and injector were 200, 250, and 230°C, respectively.

Protein Assay

Soluble protein contents of the mature seeds and germinating cotyledons were first determined using a BSA standard (Bradford, 1976). SDS-PAGE was performed according to the method of Laemmli (1970). Qualitative assay via electrophoresis was conducted on 1.5-mm-thick slab gels comprising 12% acrylamide, 0.27% bisacrylamide, 0.1% SDS, and 0.37 M tris/HCl (pH 8.8). The stacking gel consisted of 3% acrylamide, 0.08% bisacrylamide, and 0.25 M tris/HCl (pH 6.8). Protein samples (10 µg each) were dissociated in 2% SDS, 8% glycerol, and 40 mM Tris/HCl (pH 6.8). The gels were run at 30 mA, using Tris/Glycine buffer (pH 8.3), and the protein band was detected by staining with Coomassie blue and de-staining with 7% acetic acid (Laemmli, 1970).

Statistical Analysis

We used the SPSS 10.0 (SPSS, USA) statistical package program and two-way analysis of variance (ANOVA), with cultivar and salinity as the main factors, for studying these kinetics parameters. For analyzing the observed germination percentages and both seed protein and lipid contents, we ran a three-way ANOVA with salinity, cultivar, and time as the main factors. A Duncan post-hoc test was used when significant differences (at the $P < 0.05$ level) were found among salt treatments. Values were presented as the means of three independent replicates.

RESULTS AND DISCUSSION

Germination

In the salt-free (control) medium, both linseed cultivars had the same germination pattern. Untreated seeds germinated readily, their rates starting to increase progressively at 12 h after sowing (HAS), and peaking at 24 HAS (Fig. 1A, B). Maximum germination (97 to 100%) was reached at 36 HAS. Although no significant effect on final germination percentages was observed at 100 mM NaCl, the process was delayed in both cultivars, especially within the first 24 h. The highest salt treatment (200 mM NaCl) significantly inhibited and delayed germination at 72 HAS in both cultivars, this impact being more pronounced in 'H 52' (54% rate) than in 'N 51' (80%). Calculations for the parameters of germination kinetics as a function of salinity (Table 1) were consistent with the observational data (Fig. 1A, B). Indeed, while 'N 51' and 'H 52' showed similar germination characteristics in the salt-free medium, a genotype-dependent response was observed under increasing salinity. At 100 mM NaCl, the germination process was delayed; that is, both latency duration and time to 50% germination increased significantly, particularly for 'H 52'. For instance, $T_{50\%}$ increased from *ca.* 1.1 d in the control (0 mM NaCl) to 1.67 ('H 52') and 1.45 d ('N 51'). At 200 mM NaCl, the germination plateau (y_{max}) declined in both cultivars but remained significantly higher for 'N 51' (86% rate) than for 'H 52' (67%). Furthermore, the salt-related germination delay was less marked in 'N 51', and this cultivar had lower $T_{50\%}$ and t_0 values.

To assess whether moderate salinity impaired germination via osmotic and/or toxic effects, non-germinated seeds that had been treated with 200 mM NaCl were transferred to distilled water. After 7 d, the percentages of germination recovery for both cultivars were significantly lower than those obtained for the control (Fig. 1C). In addition, whereas

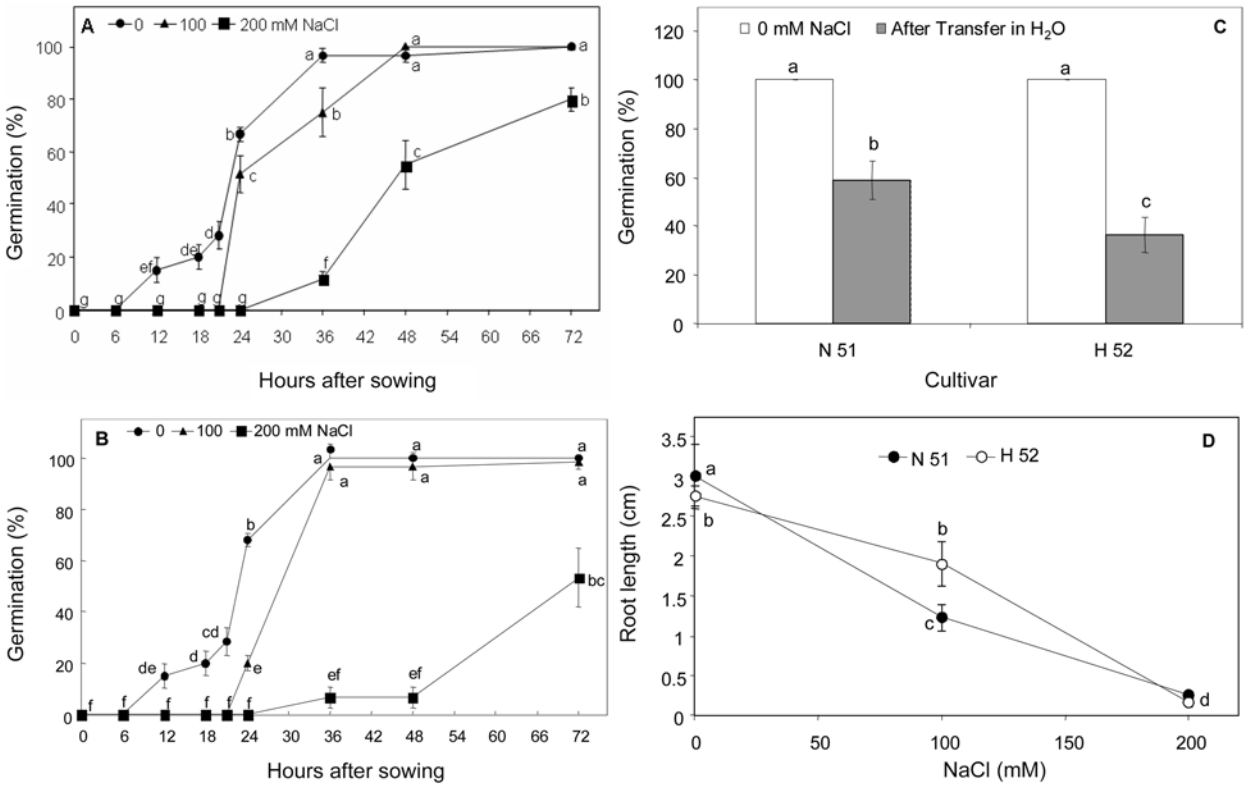

Figure 1. Time-course changes in seed germination and root elongation (%) for two salt-treated flax cultivars. Germination kinetics for 'N 51' (**A**) and 'H 52' (**B**). Symbols refer to observed percentages of germinated seeds. Germination recovery (%) after transferring non-germinated seeds (first challenged with 200 mM NaCl) to distilled water for 7 d (**C**). Root elongation (cm) of seedlings for which germination occurred under increasing salinity (**D**). (n=3). For each parameter, means followed by same letter are not significantly different at P <0.05.

Table 1. Parameters of germination kinetics as a function of salt levels in the medium. y_{max} is the number of viable seeds (% of sown), k is the probability for germination per time unit, and t_0 is the germination latency time. The time for 50% germination ($T_{50\%}$) is given as t_0 + ln(2)/k. Values for y_{max}, t_0, and k were calculated by fitting Equation [1] to the observed kinetics data. For each parameter, means (n=3) followed by at least one of the same letters are not significantly different at P <0.05.

	NaCl (mM)		
Parameter	0	100	200
N51			
y_{max} (%)	100.00a	100.00a	86.00b
k	0.99a	0.99a	0.98a
$T_{50\%}$ (d)	1.07e	1.45d	2.06b
t_0 (d)	0.37d	0.74c	1.36a
H52			
y_{max} (%)	100.00a	100.00a	67.33b
k	0.99a	0.99a	0.72b
$T_{50\%}$ (d)	1.10e	1.67c	2.35a
t_0 (d)	0.40d	0.97b	1.38a

the majority of 'N 51' seeds (*ca.* 60%) recovered their germination potential, only 36% of 'H 52' seeds remained viable. Thus, we can attribute the inhibitory effect of NaCl on flax seed germination and viability to both osmotic and toxic effects. Root elongation also decreased consistently for both cultivars as the external salt concentration increased (Fig. 1D). Similar results have been reported by Debez et al.

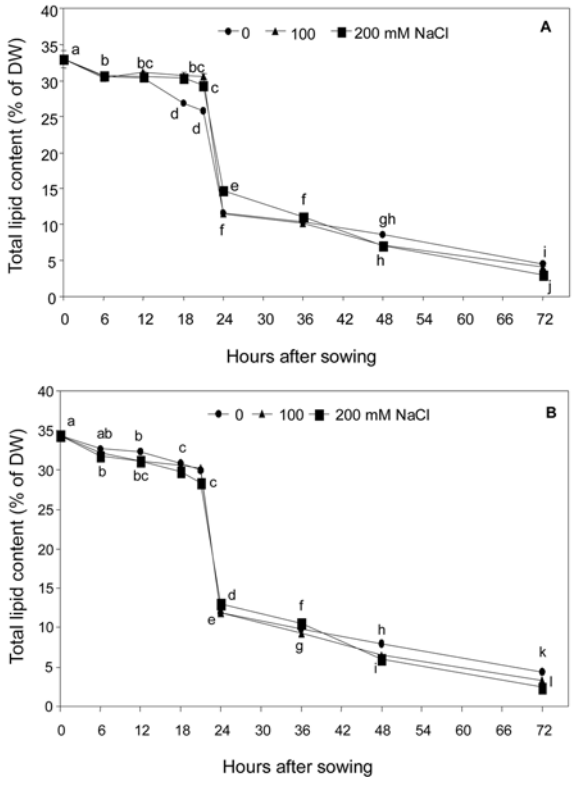

Figure 2. Time-course changes in cotyledon lipid contents (as % of DW) for salt-treated flax cultivars: N 51 (**A**) and H 52 (**B**). Means followed by same letter are not significantly different at P <0.05.

(2004), who showed that germination parameters (both capacity and kinetics) for the oilseed species *C. maritima* are delayed at NaCl concentrations lower than 200 mM NaCl, and are completely inhibited at higher levels of salinity, mainly because of osmosis (both of which are fully reversible after the seeds are transferred to water). Likewise, germination of the oilseed halophyte *Crithmum maritimum* is inhibited by the osmotic effects of salt and seawater (Atia et al., 2006). In *Brassica napus*, salinities higher than 50 mM NaCl cause a retardation of seed germination and a severe reduction in the lengths of seedling radicles (Ben Miled et al., 2000). Variability in their salt tolerance at the germination stage among and/or within crop species is of prime importance because that trait may be exploited for selecting the most desirable cultivars (Epstein et al., 1980; Shon et al., 2005). Finally, differential seed germinability has been reported in several proteo-oleaginous crops, e.g., soybean (Essa, 2002), safflower (Kaya et al., 2003), and sunflower (Ashraf et al., 2003).

Mobilization of Storage Lipids

Under non-saline control conditions, the total lipid content in 'N 51' cotyledons decreased slightly up to 18 HAS, before sharp declining by 24 HAS (Fig. 2A). This behavior was concomitant with the acceleration in its germination rate (Fig. 1A). At 72 HAS, total lipids accounted for *ca.* 4.56% of the DW. Whereas insignificant changes in the lipid degradation pattern (compared with the control) were observed at 100 mM NaCl, the highest concentration (200 mM NaCl) was associated with delayed lipid mobilization, especially from 18 to 24 HAS. During the later germination phases (36 to 72 HAS), lipid content was close to that measured from the cotyledons of seeds germinated in the presence of 0 to 100 mM NaCl. For 'H 52', the overall pattern of lipid degradation was similar, and salinity did not drastically affect this process compared with the control (Fig. 2B).

For both cultivars, linolenic (C18:3), linoleic (C18:2), and oleic (18:1) acids predominated in the seeds at the time of sowing (Table 2). In the 'N 51' control, changes in fatty acid composition of the cotyledons over time were characterized by a significant increase in both oleic and linolenic acid percentages (+12% and +25%, respectively, at 72 HAS). In contrast, the levels of palmitic (C16:0) and linoleic acids were significantly decreased. Increasing salinity resulted in a higher linolenic acid content over the germination period (+47% at 72 HAS in 100 mM NaCl), which paralleled the diminished contents of palmitic, stearic (C18:0), oleic, and

Table 2. Time-course changes in fatty acid contents (%) in cotyledons of germinating flax seeds under increasing salinity. Means (n=3) followed by at least one of the same letters are not significantly different at P <0.05.

Fatty acids, %	C16:0	C18:0	C18:1	C18:2	C18:3
N 51					
Seeds at sowing	9.8l	8.35lm	23.22h	21.05i	35.21f
0 mM NaCl 24 HAS	9.75l	8.45l	23.17h	20.98i	35.16f
36 HAS	6.82m	6.24m	23.01h	14.53j	48.26c
48 HAS	8.16l	7.35m	24.05h	21.16i	38.59e
72 HAS	8.35l	6.82m	24.01g	15.79	39.18e
100 mM NaCl 24 HAS	8.50l	6.88m	24.23h	21.14i	38.50e
36 HAS	6.62m	6.39m	25.12h	16.41j	45.28d
48 HAS	6.22m	5.35n	21.08i	15.22	51.95a
72 HAS	6.18m	5.37n	20.42i	14.98jk	52.00a
200 mM NaCl 24 HAS	6.55m	5.75mn	22.06h	14.98jk	50.95ab
36 HAS	6.13m	5.77mn	21.08i	16.12j	50.84ab
48 HAS	7.45m	6.12m	21.04i	15.16jk	47.25c
72 HAS	6.73m	6.57m	22.97h	15.81j	47.98c
H 52					
Seeds at sowing	9.95l	7.31m	24.80h	21.04i	36.9ef
0 mM NaCl 24 HAS	9.93l	8.65l	23.47h	21.41i	34.94f
36 HAS	6.94m	6.09m	22.92h	14.88jk	47.66c
48 HAS	8.51l	7.65m	24.22h	20.71i	37.41e
72 HAS	8.82l	7.85m	24.49h	15.75jk	36.17ef
100 mM NaCl 24 HAS	8.68l	6.91m	24.43h	20.87i	37.88e
36 HAS	6.98m	6.35m	24.14h	17.24j	44.26d
48 HAS	6.44m	5.41n	20.25i	14.95jk	52.51a
72 HAS	6.22m	5.77mn	20.64i	15.05jk	52.09a
200 mM NaCl 24 HAS	6.87m	5.26mn	21.18i	15.06jk	51.28ab
36 HAS	6.24m	5.90m	21.00i	15.75jk	51.09ab
48 HAS	8.97l	5.28mn	20.94i	15.75jk	46.17cd
72 HAS	6.99m	6.88m	23.27h	16.04j	46.60cd

linoleic acids. This effect was more pronounced in the latter fatty acid (-19% at 72 HAS in 100 mM NaCl). Changes in fatty acid amounts in the latter 'H 52' showed a similar pattern as a function of salinity and time.

In oilseeds, TAGs constitute the major form (>90%) of lipid reserves. Here, the TAG composition at sowing revealed six major molecular species in our two cultivars (Table 3). Linolenic and linoleic acids were predominant in the TAG spectra, with LLLn (dilinoleolinolenin) alone representing *ca.* 33% of the total. TAG molecules rich in palmitic acid (PLLn, palmitolinoleo-linolenin; PLL, palmitodilinolein; and LOP, linoleo-oleo-palmitin) accounted for another *ca.* 25%. In the 'N 51' control, germination was accompanied by the mobilization of both linoleic (PLL, LOP) and linolenic (LnOO, PLLn) acid-rich TAG molecular species. Their contents were significantly decreased (-40% on average at 72 HAS), while those of LLLn were less diminished. Similar time-related TAG qualitative changes occurred in our salt-germinating cotyledons. Except for LnOO and LOP, values at 72 HAS were close or slightly higher than those found in the control. Germination of the 'H52' control also involved TAG-degradation, with the major TAG molecular species (except LnOO) being hydrolyzed by *ca.* 33%; this trend was the same for 'N 51'.

Wanasundara et al. (1999) have shown that triacylglycerols predominate over all other lipid components in flax seed, even during the germination period. Moreover, linolenic, linoleic, and oleic acids are the primary fatty acids within all the lipid fractions of flax seed, and remain unchanged throughout germination. Therefore, the deleterious effect of salt could be ascribed to an impairment in the breakdown of seed lipids, which then diminishes the supply of soluble sugars to the growing embryonic tissues. For wheat, grain reserve mobilization decreases under greater drought and salinity (Soltania et al., 2006). In contrast, the lipid content of germinating jojoba seeds is drastically reduced in the first 10 d under moderate salinity and during the first 15 d in strong salinity (Kayani et al., 1990), likely because of a marked increase of lipase activity in those cotyledons. In sunflower, lipid content drops dramatically only in the control samples compared with just a slight, time-related decrease in the salt-treated achenes (Ashraf and Wahid,

Table 3. Time-course changes in TAG molecular species from cotyledons of germinating flax seeds under increasing salinity. Means (n=3) followed by at least one of the same letters are not significantly different at P <0.05.

TAG, %	LLLn	LLL	PLLn	LnOO	PLL	LOP
N 51						
Seeds at sowing	32.53a	10.02d	8.27d	8.85e	8.59e	7.15e
0 mM NaCl 24 HAS	32.23a	10.01d	8.62de	10.27d	8.35e	7.10e
36 HAS	32.18a	10.01d	8.59de	10.03d	8.14e	4.36f
48 HAS	33.16a	10.84d	7.21e	10.75d	3.44fg	3.04g
72 HAS	29.93b	8.95de	7.20e	4.58f	2.95g	4.24f
100 mM NaCl 24 HAS	32.84a	8.15e	7.62e	10.20d	7.71e	5.23f
36 HAS	33.24a	10.18d	7.31ef	10.65d	7.8e	4.22f
48 HAS	32.33a	10.81d	8.10e	5.19f	7.29e	4.01f
72 HAS	31.65a	9.14de	8.08e	3.31fg	4.28f	3.10g
200 mM NaCl 24 HAS	32.19a	8.97de	3.24fg	10.34d	7.11e	3.73fg
36 HAS	32.90a	9.84d	3.18fg	10.28d	4.24f	3.57fg
48 HAS	32.01a	10.66d	8.23e	3.55fg	7.16e	3.47fg
72 HAS	31.29a	9.21de	3.47fg	3.74fg	4.53f	3.51fg
H 52						
Seeds at sowing	34.04a	10.79d	10.07d	8.66de	8.85e	6.77e
0 mM NaCl 24 HAS	32.87a	9.85d	7.70e	10.37d	8.72e	5.02f
36 HAS	32.55a	9.80d	7.62e	10.32d	8.70e	5.01f
48 HAS	32.09a	10.76d	7.60e	10.80d	3.13g	2.88g
72 HAS	25.09c	8.40de	6.53ef	10.02d	2.86g	4.04f
100 mM NaCl 24 HAS	33.72a	3.58fg	8.12e	10.53d	7.29e	3.98fg
36 HAS	33.43a	2.93g	8.38e	11.13d	7.15e	3.19g
48 HAS	31.20a	10.68d	8.59de	4.08fg	8.12e	3.57fg
72 HAS	30.52a	10.39d	8.49e	3.55fg	7.28e	3.31g
200 mM NaCl 24 HAS	32.05a	9.54d	2.75g	10.20d	4.08f	3.59fg
36 HAS	31.89a	10.84d	2.05g	10.36d	3.39g	3.14g
48 HAS	31.39a	10.52d	8.13e	3.95fg	7.81e	3.23fg
72 HAS	31.78a	10.17d	3.26fg	3.26g	5.29f	3.74fg

2000; Taamalli et al., 2004). Likewise, the differential rate of breakdown in lipids under non-saline to moderate- or high-salinity conditions in oilseeds may be ascribed to salt effects on lipase activity (Huang et al., 1978). In *B. napus*, the same concentrations used in our study (100 to 200 mM NaCl) delay the degradation of triacylglycerol because of salt toxicity, which leads to the inhibition of lipase activity (Ben Miled et al., 2000). With regard to olive oil composition, Zarrouk (1999) has reported depressed levels mainly of triolein, the major TAG fraction there. However, for cotton seeds, Smaoui and Cherif (2000) have found reduced levels of linoleic acid-rich TAGs (PLL and LLL), which together account for more than 36% of the total TAG species. These conflicting results could be explained by the fact that salt stress especially affects the biosynthesis of major fatty acids, whether oleic acid in olives or linoleic acid in cotton seed. This decline in TAG synthesis may be also related to an inhibition of photosynthetic activity that is induced by high NaCl concentrations, leading to less transport of photosynthates from leaves to seeds (Smaoui and Cherif, 2000).

Protein Mobilization

In both of our flax cultivars, the germinating cotyledons showed regular and significant decreases over time in their soluble protein content (on a DW basis), irrespective of salt concentration (Fig. 3A, B). Yet, a salt-related acceleration in protein degradation was observed in both, especially at the highest salinity (200 mM NaCl). SDS-PAGE protein analysis revealed six major proteins, with molecular weights ranging from 15 to 50 kDa (Fig. 3C), in addition to a 225-kDa protein. In 'N 51' and 'H 52', no major, qualitative alterations occurred during the 0 to 48 HAS period under increasing salinity (Fig. 3D, E). At the final germination step (72 HAS), the most important changes concerned the 15- to 50-kDa proteins (less intense) as well as the induction of a 10-kDa protein. Sammour (1999) also has found six major proteins in flax (39 to 55 kDa) that represent the main seed reserves; their primary bands are for legumin-like proteins. Based on a comparison with the legumin proteins of *Pisum sativum*, this would suggest a hexameric structure for the legumin-like protein from flax.

Embryo development and protein synthesis depend on the supply of amino acids that are derived from the breakdown of storage proteins (Mayer and Poljakoff-Mayber, 1989; Bewley, 1997). Salt stress can reduce those contents in the embryo axis. For instance, in salt-stressed rice, Prakash et al. (1988) have found that protein content in the

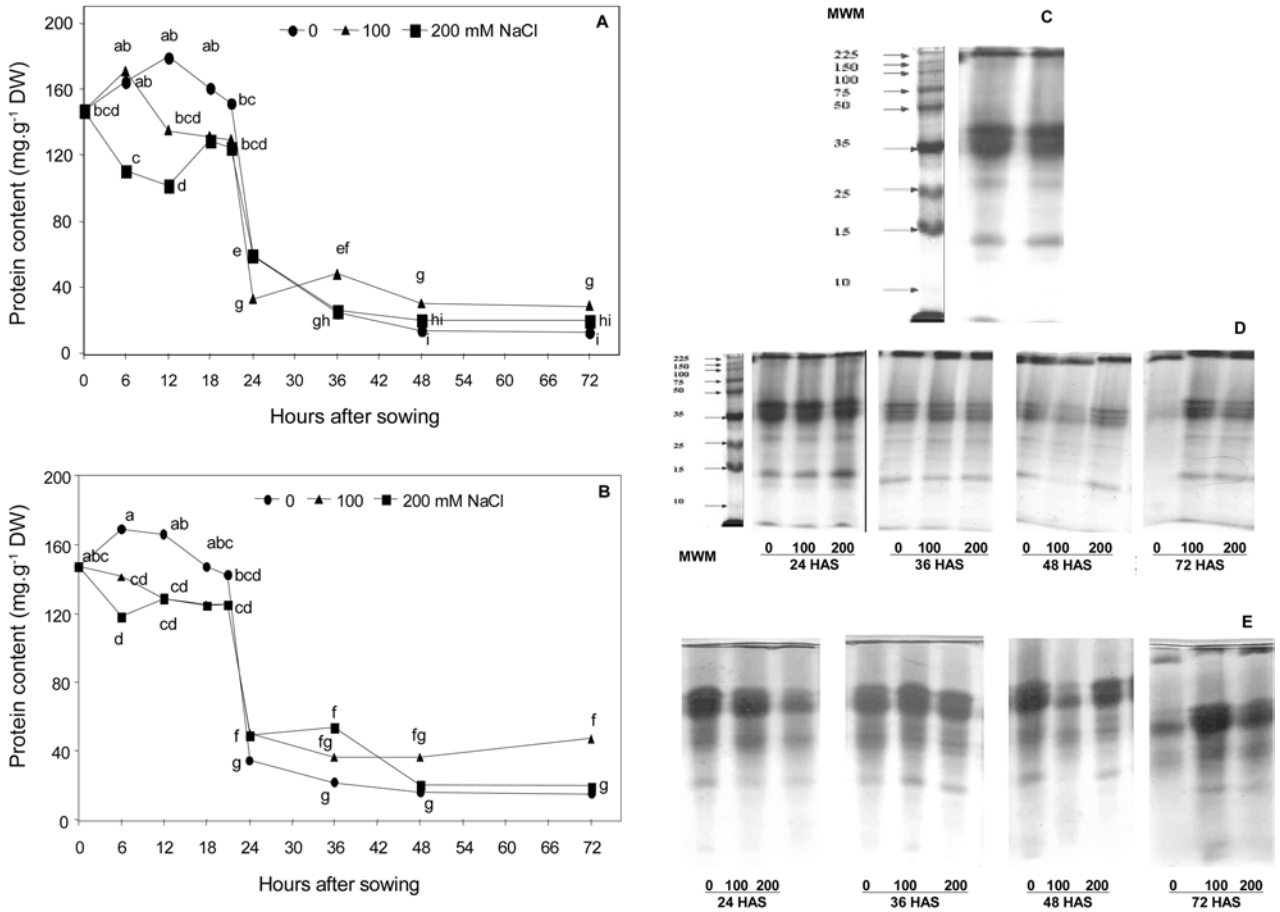

Figure 3. Quantitative time-course changes in soluble protein contents (mg g^{-1} DW) for cotyledons from salt-treated flax cultivars: 'N 51' (**A**) and 'H 52' (**B**). Means followed by same letter are not significantly different at $P < 0.05$. SDS-Polyacrylamide gel electrophoresis (12% SDS-PAGE) of soluble proteins at sowing (**C**). SDS-Polyacrylamide gel electrophoresis (12% SDS-PAGE) of different extracts from 'N 51' soluble proteins (**D**). SDS-Polyacrylamide gel electrophoresis (12% SDS-PAGE) of different extracts from 'H 52' soluble proteins (**E**). MWM, molecular weight marker. 0, 100, and 200 are NaCl concentrations (mM). HAS, hours after sowing.

shoot axis is drastically reduced, along with nucleic acids and polyamines. In sunflower, a marked decrease in soluble proteins occurs in the hypocotyls of germinating achenes (Ashraf and Wahid, 2000), probably because of the relatively lower availability of free amino acids to the hypocotyl during a time-course of extreme salt treatments. However, in agreement with our findings, both water and salt stresses stimulate starch mobilization in wheat grains by increasing the activities of β-amylase (Almansouri et al., 2001). Nevertheless, the abundance of low-molecular-weight compounds observed in our study may have resulted from the production of substances (e.g., amino acids and polyamines) that lower the intracellular osmotic potential, enabling water to move into the cells (Dantas et al., 2005).

Taken together, our results demonstrate that salinity impairs the germination of flax seed through both osmotic and toxic effects. A cultivar-dependent salt response also is present in the early developmental stages of this species. Furthermore, germination inhibition and retardation at the highest level of salinity is associated with both a delayed degradation of seed lipids during the first hours after sowing and a precocity in seed protein mobilization. Future investigations, notably those involving two-dimensional electrophoresis, should focus on the identification and comparison of specific proteins whose levels in both of these cultivars may be altered upon salinity stress.

ACKNOWLEDGEMENT

We are grateful to the "Laboratoire des Cultures Industrielles" (INRAT, Tunis) for kindly providing us with flax seeds.

Received March 15, 2007; accepted June 12, 2007.

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