



## Salt Sensitivity in *Camelina sativa* Seedlings and Polyamine Content

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### Authors' contributions

This work was carried out in collaboration between both authors. The authors of this manuscript worked together to design, conduct, analyze and interpret the findings of this experiment. Both authors read and approved the final manuscript.

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### ABSTRACT

The objective of the present investigation was to study the effect of different NaCl concentrations (0, 50, 75, 100, 125 and 150 mM) on seedlings of *Camelina sativa* (L.) Crantz. On these seedlings, we determined their appearance after 3 days of salt stress, soluble sugar and total amino acid contents and levels of polyamines. The *Camelina* seedlings suffered already at 75 mM of salt and presented more damages at higher concentrations. The content of sugars and amino acids increased up to 75 mM NaCl and then declined. The decrease was especially pronounced on soluble sugars at the highest concentrations. The Polyamine content increased at 50 mM and then decreased at higher concentration. The polyamine increase at 50 mM was mainly due to spermine. The presence of exogenous spermine (1 mM) during the stress ameliorated the tolerance of salinity, confirming previous studies about the protective role of spermine against salt stress.

**Keywords:** *Camelina sativa*; salinity; polyamines; spermine; spermidine; putrescine; soluble sugars; amino acids.

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## 1. INTRODUCTION

Salinity is the main stress that limits agricultural production. In fact, more than 20% of the cultivated area suffers from salinity and, this percentage, is increasing steadily [1]. The salt stress causes changes in various biochemical and physiological processes depending on the severity of the stress [2,3]. Salinity affects plants through both an osmotic effect (retention of water in the soil) that for a specific effect on the ion regulation [4]. Accumulation of amino acids (mainly proline) and carbohydrates play a role in mitigating the osmotic stress [5-7]. The accumulation in tissues of  $\text{Na}^+$  and  $\text{Cl}^-$  is the main detrimental effect in plants exposed to high concentrations of salt [1,8]. These ions cause severe ion imbalance and physiological disorder [1].

The polyamines (PAs) have been suggested to have a specific role in maintaining the cation-anion balance in plant cell [8]. Since they are low molecular weight polycations play an important role in ion balancing, especially when  $\text{Cl}^-$  uptake exceeds the  $\text{Na}^+$  uptake, a frequent condition under salt stress [9,10]. A countermeasure adopted by plants to resist the presence of salt is to modulate the metabolism of PAs although its significance has not yet been well elucidated [11]. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the most abundant PAs in higher plants [12]. In *Arabidopsis thaliana* (species very close to *Camelina*), a mutant lacking Spm caused hypersensitivity to NaCl, possibly due to impaired  $\text{Ca}^{2+}$ -homeostasis [13]. This phenotype was mitigated by exogenous applied spermine [14].

This study was done to test the sensitivity to salinity of *Camelina sativa* seedlings by assessing their appearance, levels of endogenous solutes (soluble sugars, amino acids) and PA levels.

## 2. MATERIALS AND METHODS

Seeds of *Camelina sativa* (L.) Crantz var. Calena were surface sterilized for 5 min with 5% (v/v) NaClO. The seeds were then spread in petri dishes (50 seeds / 10 cm diameter petri) in sterile water for germination. Seven-day-old seedlings were treated for 1 or 3 day with different salt concentrations (0, 50, 75, 100, 125 and 150 mM NaCl). The seedlings from 1 day treatment were then ground in a mortar with: a) 80% (v/v) ethanol at 50°C for 3 hours for amino acids and

soluble sugars analyses; b) 0.6 M PCA (0.1 g fresh weight [FW]/ml PCA) for PAs analysis. The homogenates were cleared by centrifugation at 15000xg for 15 min.

### 2.1 Determination of Total Amino Acids and Soluble Sugars

The level of total amino acids was determined in alcoholic-extracts by the ninhydrin method according to Moore [15] and further modified by Aurisano et al. [16]. Quantitative determination of soluble sugars was with the anthrone method [17]. To 20  $\mu\text{L}$  of alcoholic-extract were added 80  $\mu\text{L}$  of distilled water and 500  $\mu\text{L}$  of anthrone reagent (2 g  $\text{L}^{-1}$  of sulfuric acid 72%). Calibration was made with different concentrations of glucose (0, 2, 4, 6, 8 and 10  $\mu\text{g}$ ). Samples and standards were heated for 15 min at 100°C in water bath and then cooled rapidly to 0°C on ice. Samples were read at 630 nm within an hour in a plate reader Infinite 200 Pro (Tecan Italia, Cernusco sul Naviglio, Italy).

### 2.2 Determination of Polyamines

To 100  $\mu\text{L}$  of PCA extract were added 100  $\mu\text{L}$  of saturated  $\text{Na}_2\text{CO}_3$  and 200  $\mu\text{L}$  of dansyl chloride (5 mg/mL acetone). The reactions in closed tubes were stored overnight in the dark at room temperature. The dansylated PAs were extracted into 200  $\mu\text{L}$  of toluene by vortex mixing. When the two phases were well separated, 20  $\mu\text{L}$  of the toluene layer was loaded with a Linomat IV (Camag, Muttentz, Switzerland) on a HPTLC plate of silica gel 60 with concentration zone (Merck, Darmstadt, Germany). The plate was run using hexane/ethylacetate (11:8, v/v) as solvent. On removal from the tank, the plate was dried for 10 min at 50°C and the fluorescence (excitation 350 nm) of each line read into a densitometer TLC Scanner II (Camag, Muttentz, Switzerland). PAs were identified and quantitated as previously reported [18].

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Different Salt Concentrations on *Camelina* Seedlings

Seven-day-old *Camelina* seedlings (green cotyledons and 2-4 mm of root) were put in petri dishes with different NaCl concentrations (0, 50, 75, 100, 125 and 150 mM). After 3 day of stress, the appearance of seedlings was checked (Fig. 1). At higher concentrations (100-150 mM),

the seedlings were flabby, no root growth was observed and showed yellowish leaves. At 50 mM of salt, the seedlings appeared very similar to control without NaCl while, at 75 mM, they showed the first sign of cotyledons bleaching and no root growth was observed and, in general, the tissues seemed less firm.

The results indicate that 75 mM is a critical concentration of salt for seedling development in *Camelina sativa*. At this concentration, the effect observed did not probably depend on salt concentration in the growing tissues, but rather to a response to the osmolarity of the external solution [19]. Bleaching effect on cotyledons probably depends on the rate of accumulation of Na<sup>+</sup>, and on the effectiveness of Na<sup>+</sup> compartmentation within cotyledon cells [20].

### 3.2 Levels of Soluble Sugars and Total Amino Acids at Different Salt Concentrations

The levels of soluble sugars and total amino acids in the presence of different concentrations of NaCl (0, 50, 75, 100, 125 and 150 mM) are shown in Fig. 2. Both the content of sugars and amino acids were enhanced with increasing salt concentration up to 75 mM. At higher concentrations of NaCl, their levels begin to drop, and this decrease turned out stronger on soluble sugars. While the content of amino acids resulted

lower than the control (no salt) only at 150 mM NaCl, the level of soluble sugars already at 100 mM NaCl was lower than the control, continuing to fall with increasing salt concentration. The results indicate that *Camelina* seedlings are able to well counteract the increase in osmotic pressure due to the presence of salt up to 75 mM and then manifest problems at higher concentrations, starting likely to excessively consume sugars.

Sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions [21]. *Camelina* showed that, above 75 mM NaCl, is unable to maintain the sugar content (Fig. 2) even if the level of total amino acids was still high (probably for protein synthesis inhibition). Therefore, as generally observed in glycophytes [22], *Camelina* was unable to tolerate salt stress.

### 3.3 Polyamine Levels in the Presence of Different Salt Concentrations

PAs have been deemed important in preparing the plant for stress tolerance and to directly aid in ameliorating the causes of stress [23]. In Fig. 3, the level of total PAs in the presence of different salt concentrations is shown. Total PAs increased at 50 mM NaCl but then decreased, resulting below the control without salt already at 100 mM.

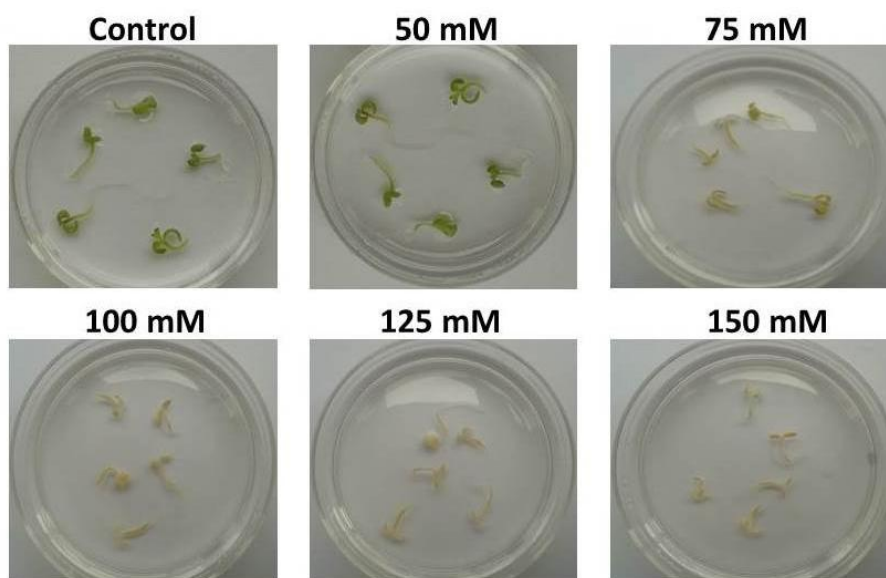


Fig. 1. Effect of 3 day treatment with different NaCl concentrations (0, 50, 75, 100, 125 and 150 mM) on *Camelina* seedlings

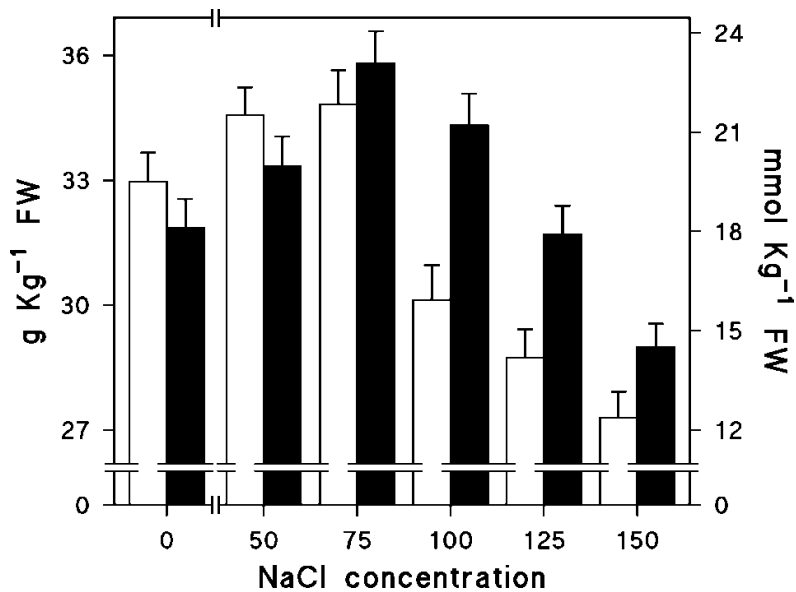


Fig. 2. Effect of different NaCl concentrations on soluble sugars (open bars) and total amino acids (solid bars). Data are expressed on a fresh weight (FW) basis

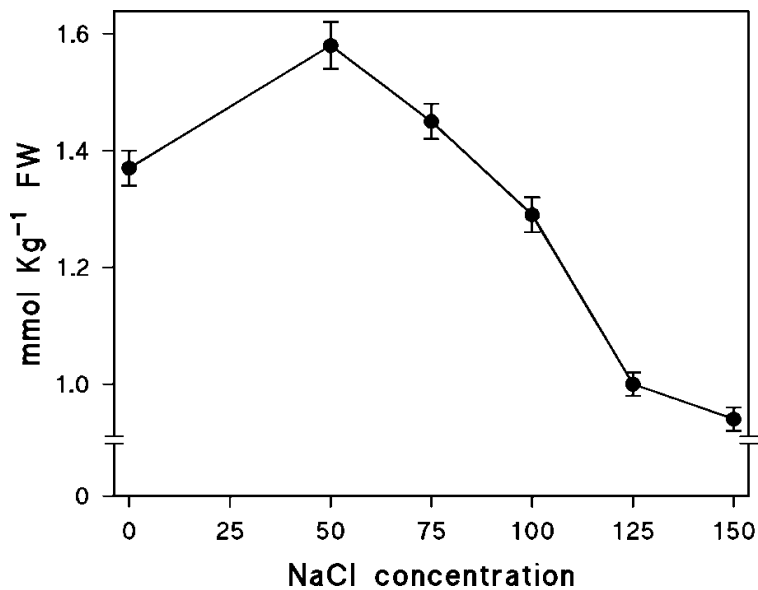


Fig. 3. Effect of different NaCl concentrations on total PA level. Data are expressed on a fresh weight (FW) basis

The PA increase at 50 mM was mainly due to the increase in Spm level (Fig. 4), while all the PA species decreased from 75 mM forward (the decrease was more pronounced on Spd and Spm levels). This causes a decline in the polyamine/diamine ratio from 3.66 at 75 mM NaCl up to 2.98 at 150 mM NaCl.

Several studies have emphasized the contribution of PAs in tolerance and/or

amelioration of stress in plants [24-28]. In particular, in *Arabidopsis* the PA Spm protected against salinity and drought stress [13,14]. Moreover, when Spm was exogenously applied, it restored a normal germination of seeds of sunflower and sorghum in the presence of NaCl [29,30]. Here, we showed that *Camelina* seedlings increased the level of Spm only at 50 mM NaCl, but then they were unable to maintain PA levels at higher salt concentrations

(Figs. 3-4). Therefore, the decline of PAs goes hand in hand with the rise of intolerance to salt stress of *Camelina* seedlings as evidenced in Fig. 1.

### 3.4 Effect of Exogenous Spm on Salinity Sensitivity of *Camelina* Seedlings

To test the importance of PAs in the tolerance of salinity, 7-day-old seedlings *Camelina* were subjected to different NaCl concentrations (0, 75

and 125 mM) in the presence or absence of 1 mM Spm (Fig. 5). The presence of exogenous Spm did not affect the control seedlings while those with 75 mM NaCl were normal and indistinguishable from the control without salt (those without Spm appeared suffering). A 125 mM NaCl, the presence of Spm kept the seedlings more green than those without Spm which were completely decoloured. The results further confirm the role of PAs and, in particular, of SPM in salt stress tolerance.

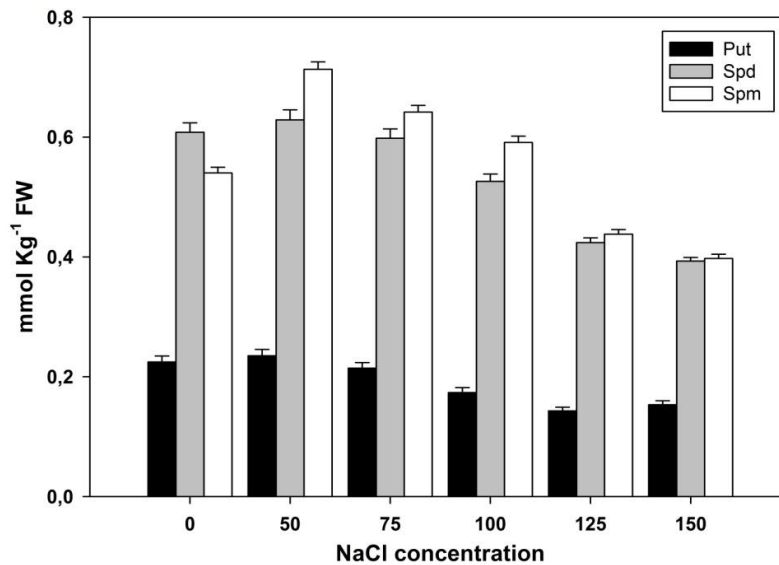


Fig. 4. Effect of different NaCl concentrations on Put, Spd and Spm levels. Data are expressed on a fresh weight (FW) basis

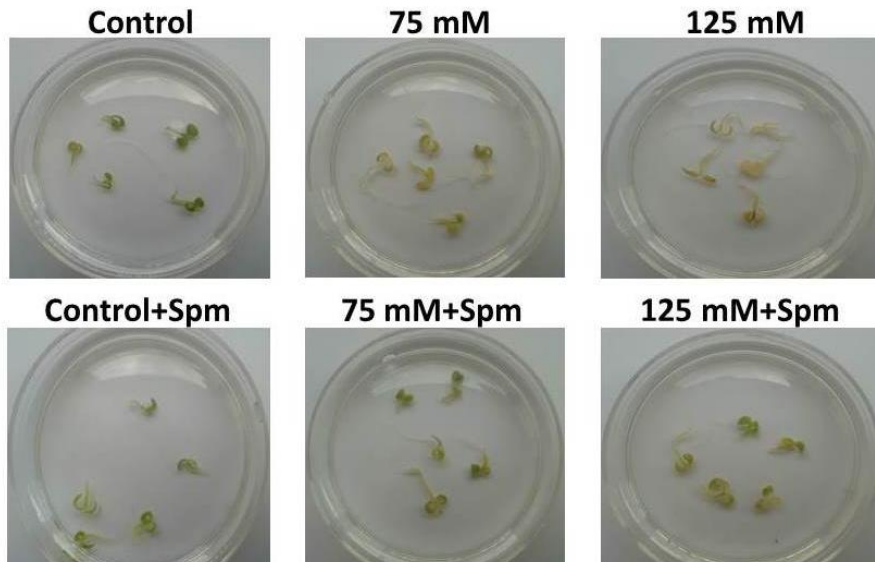


Fig. 5. Effect of 3-day treatment with different NaCl concentrations (0, 75 and 125 mM) of *Camelina* seedlings in the presence or absence of Spm (1 mM)

#### 4. CONCLUSION

*Camelina sativa* is a glycophyte sensitive to salt (NaCl) at 75 mM. At this concentration, *Camelina* is still able to counteract the osmotic pressure due to salt but begins to show damages to seedlings after 3 days of treatment. The content of PAs starts to decrease along with the appearance of damage to seedlings. In particular, the content of Spm definitely increases at 50 mM when the seedlings appear beautiful and then decreases along with the other PAs. The administration of exogenous Spm during the salt stress improved tolerance in *Camelina* seedlings. These data further support the role of Spm protection against salinity.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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