biology and life sciences forum

MDPI

Proceeding Paper

Effect of Salt Stress on Pollen Tube Growth in Two *Medicago truncatula* Ecotypes †

Adel Amar Amouri

Department of Biology, Faculty of SNV, University of Oran 1, BP 1524, Oran 31000, Algeria; amouriaa@yahoo.fr

† Presented at the 3rd International Electronic Conference on Agronomy, 15–30 October 2023; Available online: https://iecag2023.sciforum.net/.

Abstract: Annual *Medicago* species contribute significantly to improved fodder production in Algeria. The leguminous plant model is *Medicago truncatula* Gaertner. Because of its high protein content, this plant is essential for natural soil fertilization as well as good quality nutrition for animals and humans. However, abiotic stresses such as salinity are the leading cause of land degradation and crop productivity limitations worldwide, and they have an impact on legume physiology and metabolism. We investigated the pollen tube growth behavior in two contrasting *Medicago truncatula* ecotypes under salt stress (Tru 42, tolerant, and Tru 242, sensitive) with different NaCl concentrations (68, 102, and 137 mM) because pollen tube growth mechanisms can be affected by environmental stresses such as water and salt stress. According to the data, the Tru242 ecotype has a pollen tube elongation of 1.41 to 8.45 um, whereas the high pollen tube elongation of the Tru 42 ecotype is between 2.8 and 18.83 um. It is crucial to comprehend the physiological reactions of male gametophytes in order to reproduce, as salinity stress can hinder their ability to do so. It is intriguing to include the tolerant genotype in a selection program for leguminous breeding, as the analysis revealed that the tolerant ecotype has better pollen tube elongation than the sensitive one. in fact, there is not enough knowledge about pollen tolerance mechanism to salt stress of *Medicago truncatula* or other *Medicago* species.

Keywords: *Medicago truncatula*; male gametophyte; salinity; legume

1. Introduction

Annual species of the '*Medicago*' genus play an important role in improving fodder production in Algeria. They are often used in "cereal-alfalfa" rotation systems, regenerate by self-sowing, and help maintain soil fertility because of its atmospheric nitrogen-fixing capacity. Salinity is one of the environmental stresses that reduce crop productivity and quality in the world [1] and is one of the factors limiting the distribution of these plants in arid and semi-arid areas. In addition, an increase in precipitation raises saline shallow groundwater [2]. The high salt content of soil water considerably reduces the water potential of this solution and imposes water stress conditions on plants [3]. Double fertilization in flowering plants requires the targeted release of sperm from the pollen tube. After depositing on compatible stigmatic cells, the pollen germinates and the tube develops deep inside the pistil, penetrating various tissues to arrive precisely at the micropylar end of the ovule and release or deliver the sperm [4]. This mechanism can be affected by various abiotic stresses such as water stress and salinity and it is important to understand the male gametophyte physiological responses to accomplish reproduction. In this case, it is interesting to study the behavior of pollen tube development in the model leguminous of *Medicago truncatula* in order to analyze the effect of salt stress on pollen tube growth in the tolerant ecotype compared to the sensitive one because male gametophyte selection has an impact on the sporophystic stage in order to obtain an offspring of seeds tolerant to salt stress. This technique can improve legume productivity in arid land.

Citation: Amouri, A.A. Effect of Salt Stress on Pollen Tube Growth in Two *Medicago truncatula* Ecotypes. *Biol. Life Sci. Forum* **2023**, *27*, 40. https://doi.org/10.3390/IECAG2023-16381

Academic Editor: Gianni Bellocchi

Published: 29 November 2023

2. Methods

The plant material used for the morphometric study for the gametophytic phase consists of two ecotypes of the annual model species *Medicago truncatula* Gaertner (Table 1; Figure 1), provided by the National Agronomic Institute of El-Harrach (I.N.A) in Algiers-Algeria. This legume is an annual self-pollinating species characterized by a diploid complement 2n = 16. For the analysis of the male gametophyte of each ecotype, tolerant (Tru 42) and sensitive (Tru 242), pollen grains are collected from flowers at a well-defined stage, corresponding to a well-opened corolla [5]. In vitro pollen germination assay, mature pollen grains are collected from male flowers at anthesis.

Table 1. Characteristics of the two genotypes of *Medicago truncatula* used for salt stress tolerance.

Species	Ecotypes	Origin	Altitude (m)	Pluviometry (mm)	Degree of Tolerance
Medicago truncatula Gaertn.	Tru 42	Fetzara (Annaba) 36°47′50.2″ N 7°30′44.5″ E	100	660	Tolerant
	Tru 242	Kaïs (Khenchela) 35°29′30.1″ N 6°55′42.2″ E	980	450	Sensitive

Figure 1. Location of *Medicago truncatula* Gaertner in Algeria.

2.1. Pollen Condition of Germination

Pollen germination is carried out on four microscope slides, containing four media differing in their NaCl concentration, and then incubated in an oven at 25 ± 2 °C for half an hour (30 min). The observations are made under an optical microscope, objective ×10. The culture medium used is composed of 100 ppm boric acid, 100 ppm potassium carbonate, 300 ppm calcium nitrate and 10% sucrose in distilled water at pH = 6.8 [6]. The duration of pollen grains germination is 30 min under different NaCl concentrations (0; 68; 102 and 137 mM); after that, the length of the pollen tube is measured. Observations are carried out using a low magnification objective (10X) allowing the entire image to be observed. The chosen NaCl concentrations are based on a study of pollen > germination rates in different Medicago truncatula ecotypes under > salt stress compared to the sporophytic stage under the same NaCl concentrations because of the existence of structural overlap between haploid and diploid stages. The experimental device used is a block device,

staggered over time, completely random, with three repetitions (n = 3). In each replicate, each ecotype is represented by five plants.

2.2. Data Analysis

A two-factor analysis of variance test (ecotype and treatment) was performed on data obtained for pollen tube length. The relative data for each treatment, as well as those of the control, were analyzed independently by a one-way analysis of variance test (ecotype). All statistical tests were performed with Statistica software, version 7.0.

3. Results and Discussion

Pollen tube lengths without treatment were between 55.18 and 65.22 um (Figure 2). The application of salt stress reduced the growth of the pollen tube in both ecotypes compared to the control, as the salt concentration increased. This reduction varied according to ecotype and treatment (Figure 3). In this case, the Tru 42 ecotype had a high pollen tube length under different treatments and was considered the tolerant genotype, with a result between 2.8 and 18.83 um, while the Tru242 genotype was less tolerant regardless of the treatment applied and pollen tube length was between 1.41 and 8.45 um.

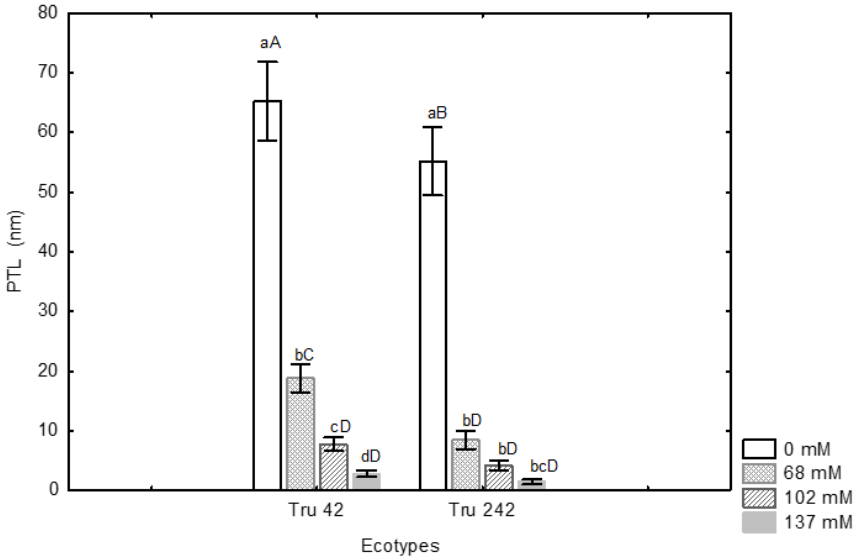

Figure 2. Pollen tube length (PTL) of two *Medicago truncatula* ecotypes under different NaCl concentrations. Bars represent average ± s.e. (n = 3). Same letters, lower case among treatments and upper case between genotypes do not significantly differ ($p < 0.05$).

After variance test analysis, ecotype effect was found to be significant at a 95% confidence level. The analysis of variance on pollen tube elongation showed a significant ecotype effect ($p < 0.01$) for the three treatments applied (68, 102 and 137 mM) of NaCl. It was noted that the treatment with 68 mM of NaCl presented the highest statistical value of F, which suggests this saline concentration discriminates best between the two ecotypes of *Medicago truncatula* studied (Table 2).

Table 2. Results of analysis of variance tests for ecotype effect by treatment for pollen tube elongation after a 30 min pollen germination in *Medicago truncatula* ecotypes.

Source of Variation	ddl	F	P
Ecotypes (Ec)	1	28.6	0.000 **
Treatment (T)	3	515.6	0.000 **
Interaction (Ec xT)	3	3.7	0.011 *

Level of significance: * = $p < 0.05$, ** = $p < 0.01$, ns: not significant.

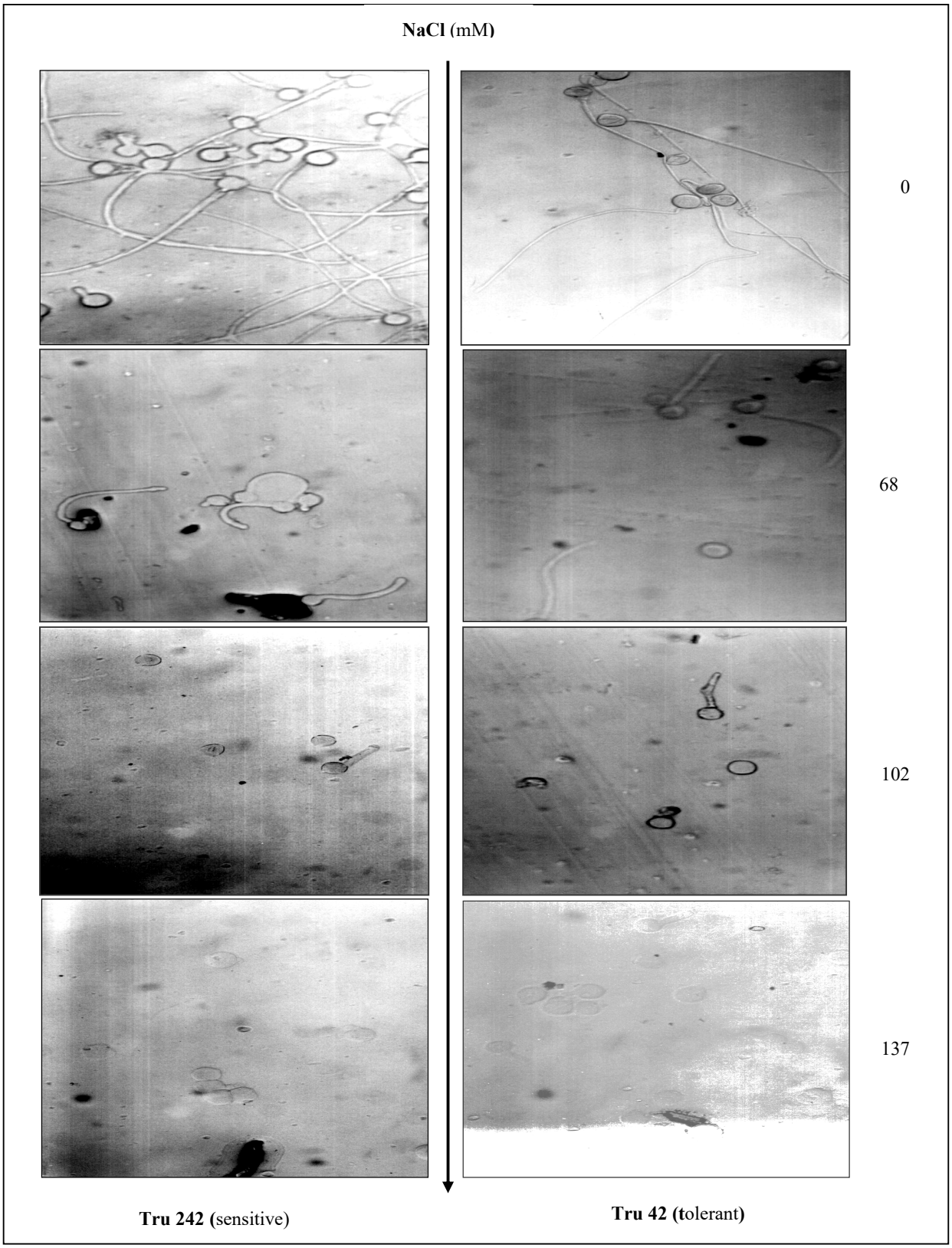

Figure 3. Some photographs of *Medicago truncatula* pollen exposed to increasing levels of salt stress.

The study of the response to salt stress of the post-germination growth of pollen grains (male gametophyte) at different concentrations of salinity induced a weak elongation of the pollen tube. The Tru 42 ecotype had a significant pollen tube elongation compared to the Tru 242 ecotype under salt stress. The analysis of genetic variability showed a significant

variation between the two contrasting ecotypes at the 68 mM NaCl concentration, and this concentration can be used to select a tolerant pollen for in vitro fecundation in *Medicago truncatula* and also in the haplodiploidisation technique for obtaining new tolerant plants to salt stress. According to [7], if there is water stress, the growth of the pollen tube inside the silk in maize is slowed down, and the pollen tube cannot reach the ovule and fertilization is not possible. This shows the importance of pollen tube elongation in the mechanism of fertilization. According to [8], NaCl reduces pollen viability in rice, and Na+ and Cl− ions are responsible for poor pollen viability, which also appears during in vitro pollen germination. An accumulation of toxic ions within pollen grains was observed also in tomato [9]. It was also reported that pollen viability decreased at flowering [10]. A study investigating the effect of salt stress on pollen grain germination ability in six ecotypes of annual *Medicago* species (*Medicago truncatula*, *Medicago ciliaris*, and *Medicago polymorpha*) showed that ecotype Tru 42 had the highest germination ability and concluded that male gametophytic selection could be an alternative for legume breeding which is more efficient and more economical compared to selection during the diploid phase [11]. In the carrot, pollen viability was also affected by salt stress [12]. Salinity can inhibit photosynthesis at a vegetative stage and induce floret sterility in relationship with decreased pollen viability [13]. Pollen salinity stress generates osmotic stress; this resistance was used as a parameter for screening tolerant ecotypes [14]. However, changes in pollen tube osmotic pressure showed that this male gametophyte can adapt to environmental stress [15,16]. It was shown that salinity decreases pollen viability in tomato during the flowering stage [17], and seed production, a key determinant of commercial success, essentially depends on pollen viability [18].

In fact, no information was available on pollen tolerance mechanism to salt stress in *Medicago truncatula* or other *Medicago* species.

4. Conclusions

The gametophytic selection study of pollen tube elongation in *Medicago truncatula* permitted the selection of the vigorous pollen from the most salinity-tolerant ecotype Tru 42. This pollen can be used to fertilize sensitive ecotypes, producing an hybrid offspring of tolerant plants introduced in arid and saline lands, used for enhancing legume productivity and natural soil fertilization by nitrogen fixation. This technique is important in legume breeding programs; it is efficient and rapid, allowing interesting agronomic character selection. Male gametophytic selection is an economical method and can be applied in other legume species. Also, it is useful in haplodiploidization technique applications.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Hasanuzzaman, M.; Fujita, M. Plant Responses and Tolerance to Salt Stress: Physiological and Molecular Interventions. *Int. J. Mol. Sci.* **2022**, *23*, 4810. [CrossRef] [PubMed]
2. Corwin, D.L.; Yemoto, K. Salinity: Electrical Conductivity and Total Dissolved Solids. *Soil Sci. Soc. Am. J.* **2020**, *84*, 1442–1461. [CrossRef]
3. Laval-Martin, D.; Mazliak, P. *Physiologie Végétale, Volume 1*; Front Cover., Danielle; Plant Physiology; Hermann: Paris, France, 1995; 539p.
4. Johnson, M.A.; Preuss, D. Plotting a course: Multiple signals guide pollen tubes to their targets. *Dev. Cell* **2002**, *2*, 273–281. [CrossRef] [PubMed]
5. Fyad-Lameche, F.Z.; Nourredine, Y.; Fairouz, L.; Larbi, A. Tolérance au froid chez la tomate (*Lycopersicum esculentum* Mill.). Etude comparée du développement du gamétophyte mâle et du sporophyte. *Acta Bot. Gallica* **2007**, *154*, 251–263. [CrossRef]

6. Vienne, D.E.D. Variabilité chez une espèce tétraploïde: Analyse iso-enzymatique et biométrique du pollen de quelques familles apparentées de luzerne. *Ann. Amélior. Plantes* **1979**, *28*, 289–307.
7. Bassetti, P.; Westgate, M.E. Senescence and receptivity of maize silks. *Crop Sci.* **1993**, *33*, 275–278. [CrossRef]
8. Khatun, S.; Flowers, T.J. Effects of salinity on seed set in rice. *Plant Cell Environ.* **1995**, *18*, 61–67. [CrossRef]
9. Ghanem, M.E.; van Elteren, J.; Albacete, A.; Quinet, M.; Martínez-Andújar, C.; Kinet, J.M.; Pérez-Alfocea, F.; Lutts, S. Impact of salinity on early reproductive physiology of tomato (*Solanum lycopersicum*) in relation to a heterogeneous distribution of toxic ions in flower organs. *Funct. Plant Biol.* **2009**, *36*, 125–136. [CrossRef] [PubMed]
10. Aref, F.; Ebrahimi-Rad, H. Physiological characterization of rice under salinity stress during vegetative and reproductive stages. *Indian J. Sci. Technol.* **2012**, *5*, 2578–2586. [CrossRef]
11. Amouri, A.A.; Fyad-Lamèche, F.Z. Comparative analysis of salinity tolerance of the male gametophyte and the sporophyte in *Medicago* at the germination stage. *Acta Bot. Malacit.* **2012**, *37*, 93–102. [CrossRef]
12. Kiełkowska, A.; Grzebelus, E.; Lis-Krzyścin, A.; Maćkowska, K. Application of the salt stress to the protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. *Plant Cell Tissue Organ Cult.* **2019**, *137*, 379–395. [CrossRef]
13. Irakoze, W.; Prodjinoto, H.; Nijimbere, S.; Rufyikiri, G.; Lutts, S. NaCl and Na2SO4 Salinities Have Different Impact on Photosynthesis and Yield-Related Parameters in Rice (*Oryza sativa* L.). *Agronomy* **2020**, *10*, 864. [CrossRef]
14. Ravikumar, R.L.; Patil, B.S.; Salimath, P.M. Drought tolerance in sorghum by pollen selection using osmotic stress. *Euphytica* **2003**, *133*, 371–376. [CrossRef]
15. Li, Y.Q.; Zhang, H.Q.; Pierson, E.S.; Huang, F.Y.; Linskens, H.F.; Hepler, P.K.; Cresti, M. Enforced growth-rate fluctuation causes pectin ring formation in the cell wall of Lilium longiflorum pollen tubes. *Planta* **1996**, *200*, 41–49. [CrossRef]
16. Biagini, G.; Faleri, C.; Cresti, M.; Cai, G. Sucrose concentration in the growth medium affects the cell wall composition of tobacco pollen tubes. *Plant Reprod.* **2014**, *27*, 129–144. [CrossRef] [PubMed]
17. Habibi, N.; Fakoor, M.Y.; Faqiri, S.M.; Sharaf, Z.; Hotak, M.S.; Danishyar, N.; Haris, M.M.; Osmani, K.S.; Shinohara, T.; Terada, N.; et al. Enhancing salinity tolerance in tomatoes at the reproductive stage by increasing pollen viability. *Revis Bionatura* **2023**, *8*, 25. [CrossRef]
18. Attioli, R.; Palombi, N.; Funck, D.; Trovato, M. Proline Accumulation in Pollen Grains as Potential Target for Improved Yield Stability Under Salt Stress. *Front. Plant Sci.* **2020**, *11*, 582877. [CrossRef] [PubMed]