Original Article

Production of tomato seedlings using seeds pelleted with *Natrum muriaticum* and submitted to saline stress

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Abstract

The development and yield of plants is directly related to the effects of salinity. There are several reports in scientific studies of significant reduction in the growth and production of tomato in soils with high electrical conductivity. The correction or recovery of salinized soils, although technically possible, is a slow and very expensive process, making it necessary to develop new technologies. The objective of this study is to evaluate the production of tomato (*Solanum lycopersicon* L.) seedlings using seeds pelleted with a homeopathic preparation of *Natrum muriaticum (Nat-m)* submitted to saline stress. The treatments consisted of the pelletization of tomato seeds with six dynamizations of *Nat-m* (3cH, 5cH, 7cH, 9cH, 11cH and 13cH). Coated or uncoated seeds (controls) were placed in phenolic foam blocks, kept in plastic trays previously moistened with 50 mM NaCl saline solution (2.922 $g.L^{-1}$ of NaCl, electrical conductivity = 4.5 dSm^{-1}) and nutrient solution (0.15 $dS.m^{-1}$) at half the ionic strength, containing 4, 1, 2, 1, 0.5, and 0.5 $mmol.L^{-1}$ of N, P, K, Ca, Mg and S, and 17.5, 9.5, 10.5, 2, 0.45 and 0.35 $mmol.L^{-1}$ of Fe, Mn, Zn, Cu and Mo, respectively. The variables evaluated were percentage of emerged plants, emergence speed index, shoot length, leaf area, number of leaves, root volume, root dry matter, and shoot dry matter. The treatments pelleted seeds/talc *Nat-m* 5cH and pelleted seeds/talc *Nat-m* 7cH increased all variables evaluated. They differed statistically from the controls, with a positive response for the development of tomato seedlings under disequilibrium conditions.

Keywords: Salinity, homeopathic preparations, *Solanum lycopersicon*, pelletization, homeostasis.

Introduction

Currently, about 1.5 million hectares of arable land are lost each year due to accumulation of salts. Salinization has been identified as the main process of soil degradation. In Brazil, although information on saline areas is not well established, it is estimated that 20-25% of irrigated areas present salinization problems [1]. In general, a soil is considered saline when the amount of salts in the soil is capable of hindering the development of plants. In most cultures, this happens when the electrical conductivity of the saturation extract is equal to or greater than 2 $dS.m^{-1}$ [2].

The interaction of salts in plants occurs directly in the enzymatic activity. Consequently, physiological and biochemical processes, such as respiration, photosynthesis, and metabolism of proteins and nucleic acids, are affected [3] promoting a high energy expenditure for the maintenance of these processes.

Plants considered sensitive to salinity do not develop or are economically viable when the electrical conductivity of the saline concentration of the medium is higher than 1.30 $dS.m^{-1}$. Moderately sensitive plants grow in solutions with an electrical conductivity between 1.30 and 3.0 $dS.m^{-1}$. Plants are moderately tolerant in a conductivity between 3.00 and 6.00 $dS.m^{-1}$, and plants are tolerant in a conductivity between 6.00 and 10.00 $dS.m^{-1}$ [4]. Tomato (*Solanum lycopersicon* L.), a vegetable greatly consumed in Brazil, is moderately sensitive to soil salinity mainly during early development [5].

The accumulation of salts in cultivated soils is quite common mainly due to high doses of fertilizers, lack of leaching of accumulated salts after cultivation, and the use of poor quality irrigation water [6]. The recovery of these soils requires high investments and demands a long time [7]. The substitution of conventional practices for ecologically correct and economically feasible practices are necessary not to impair the environment, producers, and the final product.

The use of homeopathy as an input is an approved practice. It has been growing in consequence of advances in non-conventional food production systems. It is used in several segments of agriculture, including germination [8], seedling production [9], pest control [10], plant diseases [11], increase in active substances [12], detoxification of plants due to contamination by metals such as aluminum and copper [13], and plant metabolism [14].

Ultra-diluted substances act constructively and defensively in the vitality systems of living beings. By means of self-regulation, plants respond with great intensity to homeopathy when applied in a stress situation [15]. Thus, the objective of this study was to evaluate the production of tomato seedlings (*Solanum lycopersicon* L.) using seeds pelleted with dynamizations of homeopathic preparation *Natrum muriaticum*, submitted to saline stress.

Material and Methods

Choice of treatments

Natrum muriaticum 3cH, *Natrum muriaticum* 5cH, *Natrum muriaticum* 7cH, *Natrum muriaticum* 9cH, *Natrum muriaticum* 11cH and *Natrum muriaticum* 13cH were chosen according to indications of crops that grow in soils with a tendency to salinity [15].

Preparation of inputs

Natrum muriaticum 3cH was purchased from a homeopathy pharmacy in the city of Viçosa, MG, Brazil. Other dynamizations were prepared based on this first dynamization at the Homeopathy Laboratory of the Department of Plant Science of the Federal University of Viçosa (DFT/UFV), according to the Brazilian Homeopathic Pharmacopoeia [16]. Respecting the ratio 1:100, a drop of a homeopathic substance was used in 99 drops of medium. The medium was distilled water. It was chosen in order to remove any interferences from ethanol with the results. The suctioning process was performed using a DENISE 10-50 "mechanical arm" dynamizer (AUTIC, São Paulo, Brazil). *Natrum muriaticum* 3cH, *Natrum muriaticum*

5cH, *Natrum muriaticum* 7cH, *Natrum muriaticum* 9cH, *Natrum muriaticum* 11cH e *Natrum muriaticum* 13cH were prepared prior to the coating of seeds.

Seed pelleting

The seed coating process, that is, pelletization, followed an impregnation methodology respecting the weight/volume ratio (1:1). The talc was the inert pharmaceutical talc (LAB SYNTH, Diadema, Brazil). The composition of the talc was $Mg_3Si_4O_{10}(OH)_2$, and the adhesive was the treatments (*Nat-m* 3cH, *Nat-m* 5cH, *Nat-m* 7cH, *Nat-m* 9cH, *Nat-m* 11cH and *Nat-m* 13cH) and distilled water. The homeopathic preparations and the distilled water were impregnated in a pharmaceutical talc using a petri dish in order to form an integral, white paste, which later coated the seeds.

Commercial tomato seeds (*Solanum lycopersicon* L.) were used without defensives (bare seeds). The cultivar was SANTA CLARA I 5300. The germination percentage was 90%, and the purity was 99.9% (lot 28111).

Conducting the experiment

The experiment was conducted in a greenhouse of the Department of Plant Sciences of the Federal University of Viçosa in the municipality of Viçosa, Zona da Mata Mineira. The geographical coordinates were 42°52' W and 42°50' W, and 20°44' S and 20°47' S, Brazil. The experimental design was completely randomized with eight treatments and four replications. Each experimental unit consisted of ten seeds/seedlings. The treatments are:

1- Pelleted seeds/talc + *Nat-m* 3cH
2- Pelleted seeds/talc + *Nat-m* 5cH
3- Pelleted seeds/talc + *Nat-m* 7cH
4- Pelleted seeds/talc + *Nat-m* 9cH
5- Pelleted seeds/talc + *Nat-m* 11cH
6- Pelleted seeds/talc + *Nat-m* 13cH
7- Pelleted seeds/talc + distilled water (control 1)
8- Non-pelleted seeds (control 2)

Coated or uncoated seeds (controls) were placed in phenolic foam blocks (5.0x5.0x3.8 cm), kept in plastic trays (0.80x0.40x0.10 m) previously moistened with 50 mM of NaCl saline solution (2.922 $g.L^{-1}$ of NaCl, electrical conductivity = 4.5 dSm^{-1}) and nutrient solution (0.15 $dS.m^{-1}$) [17] at half the ionic strength, containing 4, 1, 2, 1, 0.5 and 0.5 $mmol.L^{-1}$ of N, P, K, Ca, Mg and S, and 17.5, 9.5, 10.5, 2, 0.45, and 0.35 $mmol.L^{-1}$ of Fe, Mn, Zn, Cu and Mo, respectively. After 28 days of planting, the evaluations resumed.

Variables

The evaluated variables were germination percentage, germination speed index, shoot length (mm), leaf area (cm^2), number of leaves, root volume (mL), root dry matter (g), and shoot dry matter (g).

The percentage of germination was determined on the fourteenth day after sowing by computing the number of total normal seedlings per plot. The determination of the germination speed index was performed according to Maguire [18]. It is given by the sum of the ratio between the number of seeds germinated each day and the day of the evaluation according to the formula: $GSI = G_1/N_1 + G_2/N_2 + G_3/N_3 + ... + G_n/ N_n$, where: $G_1, G_2, G_3, ... , G_n$ is the number of seeds germinated on the day of observation and $N_1, N_{2, 3}, ... , N_n$ is the number of days after sowing.

The shoot length was determined using a digital caliper. The leaf area was determined using a leaf area integrator (ΔT Mater MK2 Area). The root volume was determined by difference in volume (using a 10mL beaker). Shoot dry matter of dry roots was obtained in an oven with forced ventilation at 45ºC until reaching constant weight. Data were subjected to analysis of variance and the means were compared by Tukey test at 5% probability using the SAEG 9.0 software.

Results and Discussions

The variables evaluated were germination percentage (GP), germination speed index (GSI), shoot length (SL), leaf area (LA), number of leaves (NL), root volume (RV), root dry matter (RDM), and dry shoot matter (SDM). There were significant differences by F test at 5% probability in function of treatments, thus evidencing the action of the homeopathic preparation *Natrum muriaticum* under the experimental conditions (Tables 1 and 2).

Table 1. Summary of analysis of variance of germination speed index (GSI), germination percentage (GP), leaf area (LA) and number of leaves (NL) of tomato seedlings from seeds pelleted or not with *Natrum muriaticum* submitted to saline stress.

Variation source	Degree of freedom	Mean squares			
		GSI	GP	LF	NL
Treatments	7	17.46**	1635.26**	4.059**	1.331**
Residues	24	0.28	38.54	1.077	0.032
Variation coefficient (%)		16.93	13.51	26.90	16.56

(**) Significant at 1% probability respectively with f-test.

Table 2. Summary of analysis of variance of root volume (RV), shoot length (SL), shoot dry matter (SDM) and root dry matter (RDM) of tomato seedlings from seeds pelleted or not (control) with *Natrum muriaticum* submitted to saline stress.

Variation source	Degree of freedom	Mean square			
		RV	SL	SDM	RDM
Treatments	7	1.96**	669.68**	0.0044**	0.00099**
Residues	24	0.088	13.79	0.00023	0.000055
Variation coefficient (%)		35.33	3.37	22.45	31.22

(**) Significant at 1% probability respectively with f-test.

The treatment pelleted seeds/talc + distilled water (control 1) did not differ from the treatment without pelleting (control 2) for all variables evaluated (Tables 3 and 4). Thus, there is an innocuousness of the impregnating substance (Talc PA), proving that only seed pelletization does not benefit the development of tomato seedlings.

Table 3. Mean values of germination speed index (GSI), germination percentage (GP), leaf area (cm2, LA) and number of leaves (NL) of tomato seedlings from seeds pelleted or not with *Natrum muriaticum* submitted to saline stress.

TREATMENTS	GSI	GP	LA	NL
Pelleted seeds/ Talc + *Nat-m* 3cH	1.25 **d**	25.00 **c**	3.33 **ab**	0.62 **de**
Pelleted seeds/ Talc + *Nat-m* 5cH	5.75 **a**	70.00 **a**	5.22 **a**	2.12 **a**
Pelleted seeds/ Talc + *Nat-m* 7cH	4.81 **ab**	62.50 **ab**	4.84 **a**	1.50 **b**
Pelleted seeds/ Talc + *Nat-m* 9cH	4.56 **abc**	62.50 **ab**	4.49 **ab**	1.32 **bc**
Pelleted seeds/ Talc + *Nat-m* 11cH	4.437 **bc**	57.50 **ab**	3.97 **ab**	1.20 **bc**
Pelleted seeds/ Talc + *Nat-m* 13cH	3.50 **c**	50.00 **b**	3.73 **ab**	0.95 **cd**
Pelleted seeds/ Talc + distilled water (control 1)	0.54 **d**	22.50 **c**	2.10 **b**	0.47 **e**
Non-pelleted seeds (control 2)	0.53 **d**	20.00 **c**	3.17 **ab**	0.45 **e**

Means values followed by at least a same low case letter, in the column, do not differ significantly between themselves by Tukey's test at 1% probability.

The use of *Natrum muriaticum* dynamizations in tomato seed pelletization favored the performance of GSI and GP, differing statistically from the controls 1 and 2. The treatments pelleted seeds/talc + *Nat-m* 5cH, pelleted seeds/talc + *Nat-m* 7cH, and pelleted seeds/talc + *Nat-m* 9cH had averages higher than the other treatments (Table 3) for both variables.

Marques-Silva & Bonato [19] reported the action of homeopathic medicinal products of *Rosmarinus officinalis* and *Artemisia absinthium*, in dynamizations 3cH, 6cH, 9cH, 12cH, 24cH and 30cH, on the germination and growth of common morning glory. The increase in the number of germinated seeds occurred in the 12cH, 24cH and 30cH dynamizations of *Rosmarinus*.

Table 4. Means values of root volume (mL, RV), shoot length (mm, SL), shoot dry matter (g, SDM) and root dry matter (g, RDM) of tomato seedlings from seeds pelleted or not (control) with *Natrum muriaticum* submitted to saline stress.

TREATMENTS	RV	SL	SDM	RDM
Pelleted seeds/ Talc + *Nat-m* 3cH	0.35 **cd**	16.62 **c**	0.0521 **cde**	0.0156 **bc**
Pelleted seeds/ Talc + *Nat-m* 5cH	1.87 **a**	42.22 **a**	0.1198 **a**	0.0489 **a**
Pelleted seeds/ Talc + *Nat-m* 7cH	1.77 **a**	39.97 **a**	0.0972 **ab**	0.0426 **a**
Pelleted seeds/ Talc + *Nat-m* 9cH	1.05 **b**	37.48 **a**	0.0846 **abc**	0.0321 **ab**
Pelleted seeds/ Talc + *Nat-m* 11cH	0.97 **bc**	35.06 **ab**	0.0830 **bc**	0.0200 **bc**
Pelleted seeds/ Talc + *Nat-m* 13cH	0.47 **bcd**	28.24 **b**	0.0617 **bcd**	0.0184 **bc**
Pelleted seeds/ Talc + distilled water (control 1)	0.12 **d**	11.43 **c**	0.0299 **de**	0.0075 **c**
Non-pelleted seeds (control 2)	0.10 **d**	11.17 **c**	0.0229 **e**	0.0061 **c**

Means values followed by at least a same low case letter, in the column, do not differ significantly between themselves by Tukey's test at 1% probability.

The use of seed pelletization associated with *Nat-m* 5cH and *Nat-m* 7cH promoted a significant increase by 40.22% and 43.3% in the leaf area (LA) of tomato seedlings grown in saline solution when compared with the control treatment 1 (pelletized seeds/talc + distilled water) (Table 3). According to Oliveira et al. [20], tomato plants irrigated with dam water, with an electrical conductivity of 5.7 $dS.m^{-1}$, had a 42% reduction in leaf area when compared to plants irrigated with rainwater (0.01 $dS.m^{-1}$).

For the NL variable, there was a superiority of means in the treatment pelleted seeds/talc + *Nat-m* 5cH, differing statistically from the other treatments. It promoted an increase of 22.16% and 21.22%, respectively, in the number of leaves when compared to the controls 1 and 2 (Table 3). A similar behavior was reported in field conditions in the producing region of Marília, SP. The treatment of passion fruit plants with *Silicea* 30cH promoted a 60% increase in the number of leaves, which later showed a significant correlation with the number of fruits [21].

For the RV, there was a similar behavior to the other variables mentioned above. The treatments pelleted seeds/talc *Nat-m* 5cH and pelleted seeds/talc *Nat-m* 7cH were statistically higher than the other treatments (Table 4). Several researchers have proved the use of homeopathy as an input for vegetables under stressful conditions, reporting effective results. The application of *Sulfur*, regardless of potency, was effective in the detoxification of corn plants when exposed to Al^{3+} (150 µM). The treatments *Sulfur* 5cH and *Sulfur* 30cH were effective. *Sulfur* 5cH, 12cH, 30cH, 200cH and 1McH increased by 5.3%, 6.6%, 2.7%, 12.3%, and 6.2%, respectively, the root growth compared to the control [22].

The use of seed pelletization with *Natrum muriaticum* in the 5cH, 7cH, 9cH, 11cH and 13cH dynamizations also promoted an increased shoot length (SL) of tomato seedlings subjected to saline stress, differing from the 3cH dynamization and the controls 1 and 2 (Table 4).

Pelleted seeds/talc *Nat-m* 5cH, pelleted seeds/talc *Nat-m* 7cH, and pelleted seeds/talc *Nat-m* 9cH were efficient in increasing shoot dry matter (SDM) and root dry matter (RDM) (Table 4). According to Oliveira et al. [20], the variables related to dry biomass represent the greatest effects of salinity on tomato plants.

Mechanisms and interactions were also observed in dynamized systems, such as detoxification mechanisms in seeds and plants intoxicated with heavy metals, as reported for plants of *Pisum sativum* (pea) intoxicated by copper sulphate and treated with *Cuprum sulphuricum* 15cH, a phenomenon noted also for *Cuprum sulphuricum* 14cH in *Sinapis alba* var. *Dialba* (mustard), and *Cuprum sulphuricum* 5cH in *Triticum aestivum* (wheat) [23-26].

Studies with arsenic in seeds of *Triticum aestivum* (wheat) previously subjected to lethal doses of arsenic (physiological stress) and later treated with dynamizations of arsenic trioxide (As_2O_3) 40x, 42x, 45x, non-dynamized As_2O_3, distilled water and dynamized distilled water 40x, 42x, 45x demonstrated the most promising effects on the dynamization of As_2O_3 45x and on stress. The decimal dynamizations of water were more significant when compared to the control (distilled water), and the high dilutions of non-dynamized As_2O_3 were not effective [27-29].

The development of tomato seedlings from seeds pelleted with *Natrum muriaticum* 5cH and 7cH was better for all evaluated variables, differing statistically from the controls, confirming the positive response of homeopathy in the development of seedlings under disequilibrium conditions.

Conclusion

Natrum muriaticum 5cH and 7cH cause an increase in the production of healthy tomato seedlings submitted to saline stress.

The process of seed coating using homeopathic preparations is a promising technology for the application of homeopathy to vegetables.

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