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Germination and formation of crambe seedlings irrigated with saline waters

Germinação e formação de mudas de crambe irrigadas com águas salinas

Ana Carolina F. Vasconcelos[1], Lúcia Helena G. Chaves[1], Felipe G. Souza[1], Hans R. Gheyi[1], Josely D. Fernandes[2]

[1] *Federal University of Campina Grande/Agricultural Engineering Department, Av. Aprígio Veloso, 882, CEP: 58429-140. Campina Grande, PB. Brazil, E-mails: ana3carol@yahoo.com.br, author for correspondence; lhgarofalo@hotmail.com; felipeguedesjm_16@hotmail.com; hans@agriambi.com.br;*
[2] *State University of Paraiba, Agricultural Sciences Center, Sítio Imbaúba, S/N,CEP: 58117-000, Lagoa Seca, PB. Brazil. E-mail: joselysolos@yahoo.com.br.*

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ABSTRACT

The world's growing demand for fuels and environmental concerns about the depletion of non-renewable energy reserves has aroused the interest in new options, economically and environmentally viable, as alternatives to meet this demand, as the use of biofuels. Among more than 300 oil seed plant species, only 40 have the potential for biofuel production, such as crambe (*Crambe abyssinica*). However, the effects of irrigation with saline waters in the germination of crambe seeds and development of crambe seedlings are still unknown under semiarid conditions. Considering these facts, this study aimed to evaluate the effect of salinity in seed germination and seedling development. In a germination chamber (BOD), crambe seeds were placed on a germitest paper previously moistened with NaCl solutions at the electrical conductivity (EC) levels of 0, 1.5, 3.0, 4.5, 6.0, 7, 5 and 9.0 dS m^{-1}. Four and 15 days after the set up of the assays, seed germination, number of normal seedlings, velocity of emergence of seeds, and length of seedlings and roots were evaluated. The seeds presented tolerance to the salinity levels used in this study, without presenting any decrease in the variables analyzed with the increase of ECT. The development of crambe seedlings was evaluated by placing seeds in tubes and irrigated with saline solutions (NaCl, $CaCl_2$ + NaCl) at the following EC levels: 0, 2.5, 4.0, 5.5 and 7.0 dS m^{-1}. Measurements of plant height, number of leaves and weight of fresh matter in five dates throughout the experiment were made. EC levels showed no significant effect on the analyzed parameters and there was no statistical difference between the salt types.

Key words: germination, formation of seedlings, oil seed, salinity tolerance.

RESUMO

A crescente procura mundial por combustíveis e preocupações ambientais sobre o esgotamento das reservas de energia não renováveis tem despertado o interesse em novas opções economicamente viáveis e ambientalmente corretas como alternativas para atender a essa procura, como o uso de biocombustíveis. Entre mais de 300 espécies de plantas de sementes oleaginosas, apenas 40 delas têm o potencial para a produção de biocombustíveis, como o crambe (*Crambe abyssinica*). Contudo, o efeito da irrigação com águas salinas na germinação de sementes e no desenvolvimento de mudas de crambe são ainda desconhecidos sob condições semi-áridas. O presente estudo teve como objetivo avaliar os efeitos da salinidade na germinação de sementes e no desenvolvimento de mudas de crambe. Numa câmara de germinação (BOD), sementes de crambe foram colocadas em papel germitest previamente humedecido com soluções de NaCl com condutividade elétrica (CE) de 0; 1,5; 3,0; 4,5; 6,0; 7,5 e 9,0 dS m^{-1}. Quatro e 15 dias após o início do teste foram avaliados a germinação de sementes, número de plântulas normais, velocidade de emergência e comprimento de plântulas e de raízes. As sementes de crambe apresentaram tolerância para os níveis de salinidade, não apresentando redução nas variáveis analisadas com o aumento da CE. O desenvolvimento de plântulas de crambe em tubos foi também avaliada. As sementes foram colocadas em tubos e irrigadas com soluções salinas (NaCl, $CaCl_2$ + NaCl) nos níveis de CE: 0; 2,5; 4,0; 5,5 e 7,0 dS m^{-1}. Mediu-se a altura da planta, número de folhas e peso da matéria fresca em cinco datas ao longo do ensaio. Os níveis de CE não mostraram efeito significativo sobre os parâmetros analisados e não houve diferença estatística entre os tipos de sal.

Palavras-chave: germinação, formação de mudas, sementes oleaginosas, tolerância à salinidade.

Introduction

The world's growing demand for fuels and environmental concerns about the depletion of non-renewable energy reserves has aroused the interest of developed and developing countries to consider new economically viable options and environmentally alternatives to meet this demand. Thus, new energy sources to supply fuel requirements, biofuels, have been studied (Nascimento *et al*., 2006).

The importance of biofuel is increasing due to the raising environment pollution caused by the use of fossil fuel, depletion of world petroleum reserves (petrol and diesel) and ever increasing of fossil fuel prices. Vegetable oils are an alternative form of renewable fuel to diesel engines. The main raw materials for biofuel production are cotton, peanuts, palm, sunflower, castor, jatropha, and soy. The agronomic and technological aspects observed in these raw materials for biofuel production are: oil content, yield, production system, and crop cycle, among others.

Crambe (*Crambe abyssinica* Hochst) is an oil plant of the cruciferous family native of the Mediterranean region from Ethiopia to Tanzania used for ornamental purposes and with high economical value, especially in Central Asia and the Aral Sea region (Ionov *et al*., 2013). However, it is also cultivated in tropical and subtropical regions. Crambe is an erect annual herb with large pinnate lobed leaves. Flowers are white, clustered in racemes and show the typical *Brassicaceae* structure. The spherical fruits are one-seeded and indehiscent. Despite indeterminate flowering, the fruits formed usually adhere until the later fruits mature and, even at harvest, the pericarp remains adherent to the seed. For all of these features, main seed yield components of crambe could be identify such as the number of plants per m^2, number of seed per plant and thousand kernel weight (Fontana *et al*., 1998).

It is believed that crambe has great potential as raw material for biofuel, among the 40 oil species already known with this potential (Oplinger *et al*., 1991). Crambe is usually used for oil production for industrial products, such as industrial lubricant, corrosion inhibitor and as an ingredient in the manufacture of synthetic rubber. Moreover, this crop has high productivity, good tolerance to diseases, good adaptability to different climates and growing conditions (Jasper *et al*., 2010).

After a period of great interest in Europe, including several EU projects, this crop has become partially abandoned. Significant yield variability over time and across Europe and the low value of the meal for animal feed after oil extraction restricted the marketing of crambe (Zanetti *et al*., 2013). The knowledge of crop-specific cumulative growing degree days (GDD) needed to reach maturity may allow the prediction of the best suited area for each crop, taking into account also the existing conventional cropping systems of each area. In this regard, crambe is inserted into crop rotations due to the low requirement of GDD to reach maturity (Echevenguá, 2007). Besides the great size of crambe seeds, it presents very low germination energy, capsule persistence and seed dormancy. As a result, seedling establishment can be very difficult (Berti *et al*., 2007). In Brazil, research on the culture of crambe began in 1995 in Mato Grosso do Sul (Foundation, Mato Grosso do Sul State) to evaluate its behavior in the formation of soil cover (Baez, 2007). However, with the advent of biofuel production, this oilseed has become quite interesting option for presenting yield advantages, such as earliness, tolerance to drought and frost, low production cost, yields ranging from 1000 and 1500 kg ha^{-1} and increased production of oil compared to crops such as sunflower, oilseed rape, canola, jatropha, among others. Furthermore, crambe does not compete with crops grown for food, making its cultivation feasible for biofuel production (Baez, 2007; Jasper *et al*., 2010).

The concentration of soluble salts or salinity is a limiting factor for the growth of some crops. A high salinity in irrigation water or in soil can cause problems of phytotoxicity and reduced absorption of some nutrients by the plant. The changes in metabolism induced by salinity are consequences of several physiological responses of the plant, such as changes in ionic balance, stomatal behavior and photosynthetic efficiency. The salt concentration increases in the cytoplasm and inhibits the enzyme activity of several metabolic routes and alternatively by the compartmentalization in the vacuole, the salts can be transported to the cell wall resulting in turn in dehydration of the cell. The high concentration of sodium or other cations in soil solution interfere with the physical condition of the soil or the availability of other elements, indirectly affecting plant development (Santos *et al*., 1992).

Currently, much of the planet is in a state of quantitative and qualitative water shortages, forcing prioritization of the use of waters of lower quality, which includes waters with high level of salinity. With this thought coupled with the fact that there is no specific literature about the effects of saline waters used for irrigation on crambe plants, this work was carried out in order to evaluate the effects on germination seeds of crambe in germitest paper previously moistened with saline solutions in germination chamber (BOD) and on seedling production of crambe in tubes irrigated with saline solutions.

Material and Methods

Germination of crambe seeds in germination chamber (BOD)

The germination test was carried out between June and July 2013 in a germinator chamber of BOD type with a constant temperature of 25 ºC, located in the Academic Unit of Agricultural Engineering at the Federal University of Campina Grande (UFCG), Brazil, situated at 7º 12′ 88″ S and 35º 54′ 40″ O, with an average altitude of 532 m. The seeds of crambe cultivar 'FMS Brilhante' were provided by the FMS Foundation, Maracaju Office, Mato Grosso do Sul, Brazil.

Each sample consisted of 20 seeds distributed in germitest paper substrate previously moistened with solutions of NaCl (0, 0.6, 1.6, 2.6 and 3.6 dS m^{-1}) with three replications and placed in plastic trays. The experiment followed a completely randomized design with five treatments and three replications, totaling 15 experimental units.

The quantity of each solution added to germitest paper substrate was made by adopting the procedure described in Brazil (2009), wherein the amount of water to be added is determined by the ratio of water (mL) by weight of the substrate (g). Thus, in each sample was added a volume of solution equal to 2.0-3.0 times the weight of the substrate.

For the evaluation of germination parameters, at four and 14 days after installation of the test, the first germination (%), seedling length, and germination speed index (GSI) calculated according to the formula proposed by Maguire (1962) cited by Silva *et al*. (2007) were recorded. Thus, the first germination corresponded to normal seedlings accumulated until the fourth day after sowing. Normal seedling was considered when it presented the complete radicle emergence. The Germination Speed Index (GSI) was calculated according to Carvalho *et al.* (2005) based on the following formula: GSI = (G1/N1) + (G2/N2) + ... + (Gn/Nn), where G = number of germinated seeds and N = number of days in which number of germinated seeds were observed. The seedling length was determined by measuring the normal seedlings with a ruler graduated in millimeters, and the results were expressed in cm/seedling at 14 days. Data were evaluated by analysis of variance and, when significant, the polynomial regression analysis was performed using the SISVAR 5.0 statistical software (Ferreira, 2011).

Formation of crambe seedlings irrigated with saline waters

The experiment was carried out between October and November 2013 in a greenhouse located in the Academic Unit of Agricultural Engineering at the Federal University of Campina Grande (UFCG), Brazil.

Five levels of electrical conductivity (EC) were used in the saline solutions, where the first level was correspondent to tap water (1.03 dS m^{-1}). The other levels considered in this study were 2.5, 4.0, 5.5, and 7.0 dS m^{-1}. Two types of salts, namely sodium chloride (NaCl) and sodium chloride + calcium chloride ($CaCl_2$ + NaCl), were used for the preparation of saline solutions. The experimental design was completely randomized with treatments resulting from the combination of two factors: salinity of irrigation solutions (ECs) in five levels (1.03 - control, 2.5, 4.0, 5.5, and 7.0 dS m^{-1}) and two types of salts (NaCl and $CaCl_2$ + NaCl) with twenty replications, totaling 200 experimental units.

NaCl and $CaCl_2$ were added to the public water supply in order to obtain saline solutions with different EC. The quantity of salts (Q) was determined by the equation Q (mg L^{-1}) = 640 x ECs (dS m^{-1}), as Rhoades *et al.* (2000) states, in which ECs represents the desired value of EC of the solution to be obtained. The respective solutions were stored in plastic tanks with 100 L of storage capacity.

The substrate used for the production of seedlings was the commercial substrate prepared with pine bark, namely Plantmax®. Each tube was filled with approximately 200 g of substrate, with 1.0 cm above the edge surface of the substrate to prevent loss of water during irrigation. Prior to seeding, the substrate was washed in tubes with the respective saline solutions with volume for sufficient drainage (200 mL). In each tube were sown four seeds of crambe in the approximate depth of 1.0 cm, leaving only one plant per tube after emergence. The seeds of crambe cultivar 'FMS Brilhante' were provided by the FMS Foundation, as described before. The experimental units were irrigated once a day, for maintenance of substrate moisture near field capacity.

The variables analyzed were total length of plant (aerial part + roots), number of leaves and weight of fresh matter, measured in five dates at intervals of four days between them, with the start of the measurements on the tenth day after germination. The measurements were made by selecting two random experimental units per treatment on each date, with the destruction of the experimental unit at the end of the measurements. The experiment lasted 40 days, where signs of nutritional deficiency in plants and consequent impairment of their development were observed.

Data were evaluated by analysis of variance and, when significant, the polynomial regression analysis was performed using the SISVAR 5.0 statistical software (Ferreira, 2011). For levels of salts, the data were subjected to regression analysis, while for the types of salts comparison of means was done by Tukey test at 5% level of probability.

Results and Discussion

Germination of crambe seeds in germination chamber (BOD)

Based on the results obtained in the analysis of variance (Table 1), there was no significant effect of ECs on the variables: first germination, seedling length and the germination speed index (GSI).

Increased salinity did not significantly reduce the first germination, seedling length and germination speed index at 25 ^{o}C of temperature, corroborating Fowler (1991), who presented no significant differences at 15 or 25 ^{o}C for the low salinity levels used in his study (6.3 to 17.3 dS m^{-1}). In several plant species, higher temperatures increase the detrimental effects of salinity during germination and, in a few cases, altered the optimum germination temperature at specific salinity levels. In most of the cases reviewed, however, the optimum germination temperature remained the same throughout the range of salinities tested (Francois and Kleiman, 1990; Fowler, 1991).

Based on the above findings, the salinity tolerance of crambe seeds during germination should be classified as moderately tolerant, in agreement with Fowler (1991). However, according to the salt tolerance categories established by Maas and Hoffman cited by Francois and Kleiman (1990), crambe is classified as moderately sensitive to salinity. This places crambe in the same category as all other *Cruciferae* that have been tested for salt tolerance (Campbell *et al*., 1986 cited by Francois and Kleiman, 1990).

After obtaining these results, the interest in investigating the crambe growth under more severe conditions of salinity motivated the development of the experiment previously described in this study, whose results are presented below.

Formation of crambe seedlings irrigated with saline waters

The results obtained along the experiment are presented in Table 2. No significant effects were observed in total length of plant, number of leaves, and weight of fresh matter in function of electrical conductivity of saline solutions (ECs) and types of salts (SAL).

The results obtained in this experiment are very encouraging because they indicate a great potential for crambe growth in arid and semi-arid Brazilian regions, where the water available for irrigation are considered of lower quality because of the high concentration of salts, mainly NaCl and $CaCl_2$.

The increasing importance of the use of brackish water to supplement regular irrigation has demonstrated a need for finding new potential plants with tolerance to irrigation with saline water which can be used in industrial agriculture. Ionov *et al*. (2013) studied the growth of *C. abyssinica* in arid zones and irrigated with mild saline water up to EC 6.0 dS m^{-1}, and according to these authors crambe presented tolerance to moderate salinity levels and feasibility of its culture in arid and semiarid areas with adequate salinity levels.

Although this present study did not contemplate the evaluation of oil content in crambe seeds, the literature points that salinity is not a constraint factor for it. In a study carried out by Yuldasheva *et al*. (2011) with irrigation water salinity level maintained by

Table 1 – Summary of analysis of variance for the variables: first germination, seedling length and germination speed index (GSI) in function of electrical conductivity of saline solutions (ECs).

Source	D.F.	Mean squares		
		first germination	seedling length	GSI
ECs	4	35.8 ns	5.4 ns	1.3 ns
Linear	1	0.8 ns	16.2 ns	2.6 ns
Quadratic	1	48.2 ns	4.4 ns	0.3 ns
Residual error	10	11.66	1.24	0.19
CV (%)	-	3.75	10.59	6.0

ns = non significant

Table 2 – Summary of analysis of variance for the variables (mean squares): total length of plant, number of leaves, and weight of fresh matter in function of electrical conductivity of saline solutions (ECs) and types of salts (SAL).

Source	DF	Total length of plant				
		Evaluation time				
		Date 1	Date 2	Date 3	Date 4	Date 5
ECs	4	4.65 ns	3.01 ns	3.59 ns	7.61 ns	16.83 ns
Linear	1	17.42 ns	9.51 ns	0.90 ns	21.75 ns	46.22 ns
Quadratic	1	0.21 ns	1.61 ns	2.16 ns	4.07 ns	0.07 ns
Deviation	2	0.49 ns	0.48 ns	5.65 ns	2.29 ns	10.51 ns
SAL	1	1.15 ns	1.51 ns	0.45 ns	3.36 ns	12.80 ns
ECs *SAL	4	1.99 ns	1.35 ns	1.29 ns	2.93 ns	10.26 ns
Residual error	10	3.15	3.06 ns	3.60ns	4.71 ns	41.60 ns
CV (%)		26.15	21.02	23.72	21.37	50.79
Mean		6.79	8.33	8.00	10.16	12.70
		Number of leaves				
ECs	4	0.12ns	0.37ns	1.67ns	1.30ns	0.07ns
Linear	1	0.40ns	0.22ns	1.60ns	0.90ns	0.02ns
Quadratic	1	0.00ns	0.44ns	3.50ns	1.78ns	0.02ns
Deviation	2	0.05ns	0.41ns	0.80ns	1.25ns	0.12ns
SAL	1	0.05ns	0.00ns	0.00ns	0.20ns	0.00ns
ECs *SAL	4	0.17ns	0.12ns	1.37ns	1.20ns	0.37ns
Residual error	10	0.45	1.60ns	2.00ns	2.10ns	0.70ns
CV (%)		17.89	31.62	32.89	34.50	23.24
Mean		3.75	4.00	4.30	4.20	3.60
		Weight of fresh matter				
ECs	4	0.03ns	0.03ns	0.09ns	0.12ns	0.77ns
Linear	1	0.05ns	0.10ns	0.28ns	0.15ns	0.74ns
Quadratic	1	0.06ns	0.01ns	0.01ns	0.12ns	0.35ns
Deviation	2	0.01ns	0.00ns	0.04ns	0.10ns	1.00ns
SAL	1	0.02ns	0.01ns	0.02ns	0.11ns	0.49ns
ECs *SAL	4	0.02ns	0.03ns	0.03ns	0.02ns	0.47ns
Residual error	10	0.05	0.03	0.46ns	0.41ns	0.81ns
CV (%)		38.87	54.42	74.06	58.73	79.82
Mean		0.58	0.88	0.91	1.09	1.126

adding the required amount of NaCl and $CaCl_2$ to obtain EC ranging from 1.5 to 9.0 dS m^{-1}, they observed an increase in the oil content for EC = 9.0 dS m^{-1}. Moreover, the oil content in seeds from the second-year harvest turned out to be higher than in the first year of growth. Apparently the plants adapted to the saline stress. According to these authors, the results suggest that *C. abyssinica* adapted entirely to the saline stress and produce fertile seeds containing a rather high percentage of oil.

It is worth mentioning that the results presented in this study provide strong evidence that the studies on the effects of salinity on growth of crambe under the conditions of the Brazilian semiarid region should be continued. It is known that the availability of water for irrigation with good quality in this region is very restricted, leaving only water with considerable content of salts. Thus, it is necessary the cultivation of crops more tolerant to salinity so that this region can be a producer of agricultural products for various purposes, including for the production of crops with potential for biofuel production.

Conclusions

Salinity levels of irrigation water used in this study did not affect the seed germination in BOD.
The growth of seedling crambe was not affected by the salinity. No significant interaction between the types of salts NaCl and NaCl + $CaCl_2$ and salinity levels was observed for the parameters studied.
The crambe presented salinity tolerance under the conditions provided in this study, indicating that it is a crop of great potential to be produced in the Brazilian semi-arid regions.

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