

VIGOR AND TOLERANCE OF COWPEA (*Vigna unguiculata*) GENOTYPES UNDER SALT STRESS

VIGOR E TOLERÂNCIA DE GENÓTIPOS DE FEIJÃO-CAUPI (*Vigna unguiculata*) SOB ESTRESSE SALINO

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ABSTRACT: Saline stress is a frequent phenomenon in the arid and semi-arid regions of the globe, affecting the agricultural production of these regions, and it is necessary to use strategies that minimize the impacts of saline stress under agriculture. This requires the incorporation of species, variety and genotypes tolerant to increase agricultural production in those regions. This study aimed to evaluate germination and initial growth of cowpea genotypes under salt stress. The experimental design was completely randomized in 19 x 3 factorial scheme, composed of nineteen cowpea cultivars and three osmotic potentials, with four replicates of 50 seeds each. The germination test lasted for eight days, when the seeds were evaluated for germination percentage, germination speed index, length of shoot and root, accumulation of dry mass of shoot and root. The increase in salinity affected germination and initial growth of the cowpea genotypes. The genotypes 6 - MNCO2-689F-2-8, 10 - MNCO2-675F-4-10, 12 - MNCO3-737F-5-9, 16 - MNCO2-677F-2, 18 - BRS-Pajeú and 19 – Paulistinha exhibited higher tolerance to salt stress in the stage of germination and initial growth. The genotypes 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque, 15 - MNCO3-736F-7 and 17 - BR17-Gurgueia were more susceptible to the effects of salt stress in the stage of germination and initial growth.

KEYWORDS: Seed Physiology. Germination. Water salinity.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) has great socioeconomic importance in the northeast region of Brazil, besides being one of the basic components of the diet of the northeastern population (LIMA et al., 2011). With the expansion of cowpea cultivation, new markets and commercialization perspectives open for this crop, which requires the use of new production technologies, such as irrigation, one of the agricultural technologies that have contributed the most to the increase in food production (MURTAZA et al., 2006; LIMA et al., 2011). However, its inadequate use accelerates soil salinization, especially under semi-arid conditions (ALMEIDA et al., 2012; COELHO et al., 2014).

Although sensitive to salinity, cowpea is widely spread in semi-arid regions of Northeast Brazil, where salinity problems are frequent. Hence, one alternative to improve cowpea yield would be the study of different genetic materials subjected to conditions of high saline concentration in the soil solution, to select the most adapted ones (SANTOS et al., 2009).

The osmotic effect and toxicity of ions are the main agents of salt stress on plants, but the

osmotic effect is the most damaging to germination and vigor of the plantlets, because it reduces germination speed and its uniformity, initial size and the adequate establishment of the stand (SANTOS et al., 2009; SCHEEREN et al., 2010; ALMEIDA et al., 2012). The reduction in seed vigor caused by salt stress retards, therefore, the establishment of the plantlets at the field due to the decrease in the mobilization of reserves and induction of disorders in cell membranes, caused by the increase in the osmotic pressure in the soil solution, reducing the availability of water to the seeds (BARRETO et al., 2010; ALMEIDA et al., 2012; COELHO et al., 2014).

Given the above, this study aimed to evaluate germination, initial growth and tolerance of cowpea genotypes under salt stress.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of Physiology of the Federal University of Campina Grande (UFCG), Campina Grande-PB, Brazil, from July to August 2016, using seeds of cowpea genotypes.

Twenty-six genotypes of cowpea were studied and part of them came from the Program of

Genetic Improvement of the Embrapa Mid-North (G1 - MNCO3-736F-2; G2 - MNCO3-737F-5-11; G3 - MNCO3-736F-6; G4 - MNCO2-675F-3; G5 - MNCO3-737F-11; G6 - MNCO2-689F-2-8; G7 - MNCO3-737F-5-1; G8 - MNCO3-737F-5-10; G9 - MNCO2-677F-5; G10 - MNCO2-675F-4-10; G11 - MNCO2-675F-9-5; G12 - MNCO3-737F-5-9; G13 - BRS-TUMUCUMAQUE; G14 - MNCO2-675F-9-3; G15 - MNCO3-736F-7; G16 - MNCO2-677F-2; G17 - BR17-GURGUEIA; G18 - BRS-PAJEÚ; G19 - PAULISTINHA) at three levels of osmotic potential: 0.0; -0.3 and -0.6 MPa, corresponding to water electrical conductivity (EC) values of 0.00, 8.33 and 16.67 dS m⁻¹, one EC value below and two EC values above the threshold salinity level for the crop (4.9 dS m⁻¹) (AYERS; WESTCOT, 1999), forming a 19 x 3 factorial scheme, with the resulting treatments arranged in a completely randomized experimental design, with four replicates and 50 seeds per plot.

The solutions used in irrigation were prepared through the addition of salts to distilled water, using sodium chloride (NaCl), which accounts for 70% of the salt ions in water sources used for irrigation in small properties of Northeast Brazil (MEDEIROS et al., 2003).

The seeds were put in rolls of Germitest[®] paper, moistened until 2.5 times their dry weight, according to the treatment, and placed to germinate in a *Biochemical Oxygen Demand* (B.O.D.) chamber, at 25 °C and with photoperiod of 8 hours of light and 16 hours of darkness. The germination test lasted for eight days (BRASIL, 2009).

During the experiment, the germination of cowpea seeds was monitored through daily counts of the number of germinated seeds, i.e., with production of the first radicle, without discarding them, thus obtaining a cumulative value. Hence, the number of emerged plantlets referring to each count was obtained by subtracting the reading of the previous day from the value of the current day. Then, the number of germinated seeds referring to each count was used to calculate the germination speed index (GSI), based on the equation described by Maguire (1962):

$$GSI = \frac{G_1 + G_2 + \dots + G_n}{(N_1) + (N_2) + \dots + (N_n)}$$

Where: GSI = germination speed index; G = Number of germinated seeds in each count; N = number of days from sowing to each count.

After the germination test, at eight days after sowing, the percentage of normal plantlets (PNP) (%) was determined based on the relationship between the number of germinated seeds and the number of seed sown. Also at the end of the

germination test, the primary root and the shoots of normal plantlets of each replicate were measured using a ruler graduated in millimeter and the results were expressed in cm plantlet⁻¹. Shoot dry matter (SDM) and radicle dry matter (RDM) were determined by cutting the plantlets and storing the different portions in Kraft paper bags, which were dried in a forced-air oven at 65 °C until constant weight and weighed on an analytical scale (0.0001 g), with results expressed in g plantlet⁻¹ (NAKAGAWA, 1999).

The obtained data were subjected to analysis of variance by F test. In case of significance, the Scott-Knott means grouping test was applied for the factor genotype and the Tukey test was applied for the factor salinity, both at 0.05 significance level, using the statistical software SISVAR[®] (FERREIRA, 2011).

RESULTS AND DISCUSSION

There was significant interaction ($p < 0.05$) between the salinity levels and studied genotypes for the variables: germination percentage, germination speed index, shoot length, radicle length, shoot dry matter and radicle dry matter (Tables 1, 2 and 3).

The germination percentage of the cowpea genotypes 4 - MNCO2-675F-3, 7 - MNCO3-737F-5-1, 9 - MNCO2-677F-5, 10 - MNCO2-675F-4-10, 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque, 14 - MNCO2-675F-9-3 and 15 - MNCO3-736F-7 was reduced from 36 to 18%, due to the decrease in the osmotic potential from 0.0 MPa to -0.6 MPa (Table 1). Additionally, at the osmotic potential of -0.3 MPa, only the genotypes 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque and 15 - MNCO3-736F-7 showed significantly reduced ($p < 0.05$) germination percentage, which indicates lower degree of tolerance of these genotypes in the germination stage (Table 1).

The germination speed index of the genotypes 4 - MNCO2-675F-3, 9 - MNCO2-677F-5, 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque and 15 - MNCO3-736F-7 was also reduced by the increase in salinity, corroborating the results of germination percentage, confirming a higher sensitivity of these genotypes to salt stress (Table 1). The germination percentage and germination speed index of the genotypes 3 - MNCO3-736F-6, 5 - MNCO3-737F-11, 8 - MNCO3-737F-5-10, 12 - MNCO3-737F-5-9, 16 - MNCO2-677F-2, 17 - BR17-Gurgueia, 18 - BRS-Pajeú and 19 - Paulistinha were not influenced by the reduction in the osmotic potential from 0.0 MPa to -0.6 MPa

(Table 1), which denotes higher degree of tolerance of these genotypes in the germination stage.

Table 1. Percentage of germination (PG) and germination speed index (GSI) of cowpea genotypes under saline stress.

Genótipos	PG (%)			GSI		
	Osmotic potential (MPa)					
	0.0	-0.3	-0.6	0.0	-0.3	-0.6
1 - MNCO3-736F-2	80Ab	66Ac	67Ac	9.65Ab	6.51Bc	7.08Bc
2 - MNCO3-737F-5-11	80Ab	72Ab	68Ac	8.60Ac	8.23Ab	6.68Bc
3 - MNCO3-736F-6	94Aa	96Aa	95Aa	11.58Aa	11.12Aa	10.44Aa
4 - MNCO2-675F-3	93Aa	79ABb	70Bc	8.30Ac	5.77Bc	5.89Bd
5 - MNCO3-737F-11	98Aa	94Aa	95Aa	12.15Aa	11.54Aa	10.61Aa
6 - MNCO2-689F-2-8	94Aa	80Ab	83Ab	7.11Ad	5.64Abc	5.12Be
7 - MNCO3-737F-5-1	93ABa	96Aa	79Bb	10.82Ab	9.58Ab	10.90Aa
8 - MNCO3-737F-5-10	100Aa	100Aa	98Aa	12.32Aa	12.50Aa	11.66Aa
9 - MNCO2-677F-5	74Ab	72Ab	55Bd	6.88Ad	4.19Bd	3.36Bf
10 - MNCO2-675F-4-10	84Ab	71AB	65Bc	5.08Ae	4.16Ad	4.03Ae
11 - MNCO2-675F-9-5	86Ab	60Bc	40Cd	4.60Ae	5.19Ac	2.75Bf
12 - MNCO3-737F-5-9	93Aa	88Aa	87Ab	11.43Aa	11.04Aa	10.15Aa
13 - BRS-Tumucumaque	79Ab	52Bc	61Bc	8.52Ac	4.93Bd	4.71Be
14 - MNCO2-675F-9-3	88Aa	75ABb	62Bc	3.94Ae	4.53Ad	3.10Af
15 - MNCO3-736F-7	78Ab	59Bc	56Bd	8.75Ac	5.53Bc	4.50Be
16 - MNCO2-677F-2	96Aa	92Aa	91Aa	7.85Ac	8.43Ab	7.37Ac
17 - BR17-Gurgueia	97Aa	95Aa	94Aa	12.08Aa	11.55Aa	11.20Aa
18 - BRS-Pajeú	92Aa	94Aa	83Ab	7.27ABd	8.17Ab	5.85Bd
19 - Paulistinha	100Aa	98Aa	89Ab	10.61Ab	9.22Ab	8.95Ab
CV(%)	11.31			11.86		

Equal lowercase letters in the column do not differ from the Scott and Knott test ($p < 0.05$), and the same upper case letters in the row do not differ from the Tukey test ($p < 0.05$).

Similar results have been reported by Santos et al. (2009), Almeida et al. (2012) and Scheeren et al. (2010), who evaluated salt stress on the germination of bean and soybean seeds, respectively, and observed that the osmotic effect compromises the germination and vigor of the plantlets, because it reduces germination speed and uniformity, initial size and the adequate establishment of the stand, because of the damage on physiological and biochemical functions due to the lower hydration of the tissues.

The reduction in the osmotic potential of the substrate from 0.0 MPa to -0.6 MPa did not significantly influence ($p < 0.05$) the shoot length of the genotypes 6 - MNCO2-689F-2-8, 9 - MNCO2-677F-5, 12 - MNCO3-737F-5-9, 13 - BRS-Tumucumaque, 15 - MNCO3-736F-7 and 18 - BRS-Pajeú (Table 2). The reduction of growth is one of

the most evident effects of the salt stress on plants, because, during the absorption of water, they concomitantly absorb Na^+ and Cl^- ions, which in excess in the protoplasm cause ionic disorder and act on enzymes and membranes (TAIZ; ZEIGER, 2013). However, the increase in salinity negatively affected the shoot growth of the genotypes 1 - MNCO3-736F-2, 3 - MNCO3-736F-6, 4 - MNCO2-675F-3, 5 - MNCO3-737F-11, 8 - MNCO3-737F-5-10, 11 - MNCO2-675F-9-5 and 17 - BR17-Gurgueia, with reductions on the order of 69.88, 65.22, 80.38, 68.51, 63.29, 71.14 and 82.82% in the comparison between the potentials of 0.0 and -0.6 MPa, respectively (Table 2).

When subjected to the osmotic potential of -0.6 MPa, the radicle growth of all studied cowpea genotypes was reduced, compared with the genotypes subjected to the control treatment (0.0

MPa) (Table 2). The reduction in root system is an important mechanism of tolerance under saline conditions, because it limits the entry of water and, consequently, the entry of salts, thus avoiding toxicity by specific ions (MUNS; TESTE, 2008; Oliveira et al., 2016; Sá et al., 2016). However, when subjected to intermediate conditions of salinity (-0.3 MPa), only the genotypes 6 - MNCO2-689F-2-8, 13 - BRS-Tumucumaque, 15 - MNCO3-

736F-7, 16 - MNCO2-677F-2, 18 - BRS-Pajeú and 19 - Paulistinha exhibited no influence of the increase in salinity on radicle growth (Table 2). This can be related to the higher degree of tolerance of these genotypes, because the genotypes 6 - MNCO2-689F-2-8 and 18 - BRS-Pajeú also showed no influence of the increase in salinity on germination and shoot growth (Tables 1 and 2).

Table 2. Length of aerial part (LAP) and radicle (LR) of cowpea genotypes under saline stress.

Genótipos	LAP (cm)			LR (cm)		
	Osmotic potential (MPa)					
	0.0	-0.3	-0.6	0.0	-0.3	-0.6
1 - MNCO3-736F-2	3.52Ab	1.84Bc	1.06Ca	3.27Ad	2.28Bd	2.00Ba
2 - MNCO3-737F-5-11	1.95Ad	1.70Ac	0.72Bb	2.03Ae	0.97Be	0.90Bb
3 - MNCO3-736F-6	3.48Ab	1.97Bc	1.21Ca	4.38Ac	2.25Bd	1.26Cb
4 - MNCO2-675F-3	3.62Ab	1.85Bc	0.71Cb	6.05Ab	2.15Bd	1.72Ba
5 - MNCO3-737F-11	3.62Ab	1.89Bc	1.14Ca	4.65Ac	2.69Bc	2.10Ba
6 - MNCO2-689F-2-8	1.57Ad	1.52Ac	1.35Aa	1.63Ae	1.21ABd	0.71Bb
7 - MNCO3-737F-5-1	1.97Ad	1.19Bd	0.88Bb	3.51Ad	0.97Be	1.09Bb
8 - MNCO3-737F-5-10	3.76Ab	2.55Bb	1.38Ca	6.62Aa	2.84Bc	1.81Ca
9 - MNCO2-677F-5	1.65Ad	1.07Ad	1.04Aa	2.35Ae	0.70Be	0.57Bb
10 - MNCO2-675F-4-10	4.07Ab	1.58Bc	1.11Ba	6.62Aa	3.00Bc	2.21Ba
11 - MNCO2-675F-9-5	2.98Ac	1.54Bc	0.86Cb	5.49Ab	2.97Bc	0.89Cb
12 - MNCO3-737F-5-9	1.91Ad	1.69Ac	1.39Aa	3.08Ad	1.46Be	0.76Bb
13 - BRS-Tumucumaque	1.63Ad	1.26A	1.15Aa	1.71Ae	0.94ABe	0.80Bb
14 - MNCO2-675F-9-3	1.55Ad	1.12Ad	0.69Bb	1.41Ae	0.75Be	0.71Bb
15 - MNCO3-736F-7	1.46Ad	1.20Ad	1.11Aa	1.39Ae	1.40Ae	1.08Bb
16 - MNCO2-677F-2	1.78Ad	1.64Ac	0.95Bb	1.69Ae	2.49ABd	0.83Bb
17 - BR17-Gurgueia	5.59Aa	3.44Ba	0.96Cb	5.77Ab	3.28Bb	1.46Cb
18 - BRS-Pajeú	1.80Ad	1.75Ac	1.42Aa	3.02ABd	4.25Aa	2.41Ba
19 - Paulistinha	1.98Ad	1.57Ac	0.52Bb	1.69ABe	2.18Ad	0.85Bb
CV (%)	11.83			13.91		

Equal lowercase letters in the column do not differ from the Scott and Knott test ($p < 0.05$), and the same upper case letters in the row do not differ from the Tukey test ($p < 0.05$).

The shoot dry matter accumulation of the genotypes 2 - MNCO3-737F-5-11, 6 - MNCO2-689F-2-8, 7 - MNCO3-737F-5-1, 12 - MNCO3-737F-5-9 and 19 - Paulistinha decreased significantly ($p < 0.05$) with the reduction in the osmotic potential of the substrate from 0.0 MPa to -0.6 MPa (Table 3). However, the genotypes 11 - MNCO2-675F-9-5, 15 - MNCO3-736F-7 and 17 - BR17-Gurgueia increased the accumulation of shoot dry matter due to the reduction in the osmotic potential of the substrate from 0.0 MPa to -0.6 MPa, which is related to the small growth and, consequently, the non-consumption of seed

reserves, corroborating the low dry matter accumulation in the radicle. Thus, the higher accumulation of dry matter is related to the seed and not to the plantlet (Tables 2 and 3). However, the reduction in seed vigor caused by the salt stress, retards the establishment of the plants at field and is due to the decrease in the mobilization of reserves and induction of disorders in the cell membranes, caused by the increase in the osmotic pressure of the soil solution, reducing the availability of water to the seeds (BARRETO et al., 2010; ALMEIDA et al., 2012; COELHO et al., 2014).

Table 3. Dry shoot mass (DSM) and radicle (DRM) of cowpea genotypes under saline stress.

Genótipos	DSM (g)			DRM (g)		
	Osmotic potential (MPa)					
	0.0	-0.3	-0.6	0.0	-0.3	-0.6
1 - MNCO3-736F-2	0.591Ac	0.462Ad	0.615Ab	0.011Ad	0.012Ac	0.009Ab
2 - MNCO3-737F-5-11	0.793Ab	0.615Bc	0.545Cc	0.018Ac	0.004Bd	0.006Bb
3 - MNCO3-736F-6	0.637Ac	0.615Ac	0.718Aa	0.013ABd	0.018Ac	0.009Bb
4 - MNCO2-675F-3	0.545Ac	0.547Ac	0.647Ab	0.032Ab	0.021ABb	0.016Ba
5 - MNCO3-737F-11	0.640Ac	0.635Ac	0.673Aa	0.022Ac	0.016ABc	0.013Bb
6 - MNCO2-689F-2-8	0.999Aa	0.719Bb	0.768Ba	0.018Ac	0.009AB	0.006Bb
7 - MNCO3-737F-5-1	0.727Ab	0.600ABc	0.538Bc	0.022Ac	0.003Bd	0.004Bb
8 - MNCO3-737F-5-10	0.564Bc	0.550Bc	0.710Aa	0.034Ab	0.010Bd	0.014Ba
9 - MNCO2-677F-5	0.641Ac	0.739Ab	0.765Aa	0.020Ac	0.010Bd	0.012Bb
10 - MNCO2-675F-4-10	0.631Bc	0.716ABb	0.858Aa	0.050Aa	0.021Bb	0.025Ba
11 - MNCO2-675F-9-5	0.565Bc	0.706ABb	0.749Aa	0.035Ab	0.013Bc	0.008Bb
12 - MNCO3-737F-5-9	0.830Aa	0.594Bc	0.542Bc	0.030Ab	0.007Bd	0.003Bb
13 - BRS-Tumucumaque	0.722Ab	0.736Ab	0.786Aa	0.006Ad	0.010Ad	0.007Ab
14 - MNCO2-675F-9-3	0.901Aa	0.814Aab	0.826Aa	0.030Ab	0.014Bc	0.007Bb
15 - MNCO3-736F-7	0.685Bc	0.696Bb	0.832Aa	0.005Ad	0.007Ad	0.004Ab
16 - MNCO2-677F-2	0.767Ab	0.880Aa	0.737Aa	0.013AB	0.020Ab	0.006Bb
17 - BR17-Gurgueia	0.304Bd	0.322ABe	0.473Ac	0.017Ac	0.026Ab	0.018Aa
18 - BRS-Pajeú	0.730Ab	0.775Ab	0.641Ab	0.018ABc	0.030Aa	0.012Bb
19 - Paulistinha	0.865ABa	0.953Aa	0.731Ba	0.017Ac	0.019Ac	0.007Bb
CV (%)	13.38			12.38		

Equal lowercase letters in the column do not differ from the Scott and Knott test ($p < 0.05$), and the same upper case letters in the row do not differ from the Tukey test ($p < 0.05$).

Regarding the plantlets subjected to salt stress, vigor is more affected than germination, causing greater reduction of biomass, which can be attributed to the decrease in cell division and expansion, leading to a loss of yield by the plant (SANTOS et al. 2009; ALMEIDA et al., 2012; LOPES et al., 2014; BERNARDES et al., 2015). However, some materials exhibit better performance under salt stress conditions, as observed in the genotypes 4 - MNCO2-675F-3, 8 - MNCO3-737F-5-10, 10 - MNCO2-675F-4-10 and 17 - BR17-Gurgueia, which showed the highest accumulation of radicle dry matter at the osmotic potential of -0.6 MPa, in relation to the other studied genotypes (Table 3).

The genotypes 1 - MNCO3-736F-2, 13 - BRS-Tumucumaque, 15 - MNCO3-736F-7 and 17 - BR17-Gurgueia were not influenced by the increase in salinity, but they showed the lowest accumulations of dry matter, even in the control

treatment (0.0 MPa). This indicated lower development and probably consumption of seed reserves for plantlet growth, which is consistent with the results for shoot dry matter (Table 3).

CONCLUSIONS

The increase in salinity affects germination and initial growth of the cowpea genotypes.

The genotypes 6 - MNCO2-689F-2-8, 10 - MNCO2-675F-4-10, 12 - MNCO3-737F-5-9, 16 - MNCO2-677F-2, 18 - BRS-Pajeú and 19 - Paulistinha are the most tolerant to the salt stress in the stage of germination and initial growth.

The genotypes 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque, 15 - MNCO3-736F-7 and 17 - BR17-Gurgueia are the most sensitive to salt stress in the stage of germination and initial growth.

RESUMO: O estresse salino é um fenômeno frequente em regiões áridas e semiáridas do globo, afetando a produção agrícola dessas regiões, sendo necessário lançar mão de estratégias que minimizem os impactos do estresse salino sob agricultura, para isso é necessário a incorporação de espécies, variedade e genótipos tolerantes para aumentar a produção nessas regiões. Assim, objetivou-se com esse trabalho avaliar a germinação e o crescimento inicial de genótipos de feijão-caupi sob estresse salino. Utilizou-se de um delineamento experimental inteiramente casualizado em esquema fatorial 19 x 3, constituído de dezenove cultivares de feijão-caupi e três potenciais osmóticos, com quatro repetições de 50 sementes cada. O teste de germinação teve duração de oito dias, quando as sementes foram avaliadas a quanto à percentagem de germinação, índice de velocidade de germinação, comprimento da parte aérea e da raiz, acúmulo de massa seca da parte aérea e da raiz. O aumento da salinidade afetou a germinação e o crescimento inicial dos genótipos de feijão-caupi. Os genótipos 6 - MNCO2-689F-2-8, 10 - MNCO2-675F-4-10, 12 - MNCO3-737F-5-9; 16 - MNCO2-677F-2; 18 - BRS-Pajeú e 19 - Paulistinha apresentaram maior tolerância ao estresse salino na fase de germinação e crescimento inicial. Os genótipos 11 - MNCO2-675F-9-5, 13 - BRS-Tumucumaque; 15 - MNCO3-736F-7 e 17 - BR17-Gurgueia foram mais suscetíveis aos efeitos do estresse salino na fase de germinação e crescimento inicial.

PALAVRAS-CHAVE: Fisiologia de Sementes. Germinação. Salinidade da água.

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