PHYSIOLOGICAL RESPONSE OF COWPEA SEEDS TO SALINITY STRESS[1]

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ABSTRACT- This work aimed to evoluate physiological response of cowpea (*Vigna unguiculata* L. Walp) seeds submitted to salt stress. Seeds of cultivars 'Epace-10'; 'Canapu' and 'Pitiúba' of cowpea, were submitted to germination test in germinator at 25^{0}C, in "germitest" papers imbibed in distilled water or in 0, 10, 50, 100 and 200mol m^{-3} NaCl solutions. At the first and second counting of the germination test, normal seedlings were accounted, weighted and dried, obtaining data for vigor, total germination, fresh matter weight and dry matter weight. The seedlings hypocotyls, root and total length were measured total proteins content in cotyledons were obtained from germinating seeds. The presence of salt at concentrations higher than 50mol m^{-3} NaCl affect the germination, seedlings growth and cotyledons total protein synthesis of all cowpea cultivars. The seeds of cultivar pitiúba were is more tolerant to salinity, than the cultivars Canapu and Epace-10.

Index terms: *Vigna unguiculata,* sodium chloride, germination, vigor, protein

RESPOSTA FISIOLÓGICA DE SEMENTES DE FEIJÃO CAUPI AO ESTRESSE SALINO

RESUMO - Este trabalho objetivou avaliar a resposta fisiológica de sementes de três cultivares de feijão caupi (*Vigna unguiculata* L. Walp) ao estresse salino. As sementes das cultivares 'Epace-10'; 'Canapu' e 'Pitiúba' foram submetidas ao teste de germinação em germinador a 25^{0}C, em papel "germitest" embebido em água destilada ou em soluções de NaCl nas concentrações de 0, 10, 50, 100 e 200mol m^{-3}. Na primeira e segunda contagem do teste de germinação, as plântulas normais foram contadas, pesadas e secadas, obtendo-se dados de vigor, germinação, massa de matéria fresca e massa de matéria seca. O comprimento do hipocótilo, raiz e total foram medidos e o teor de proteínas totais nos cotilédones foi obtido em plântulas de 3, 5 e 8 dias. As concentrações de NaCl acima da 50mol m^{-3} afetam a germinação e o vigor das sementes, crescimento de plântulas, bem como a síntese de proteínas totais. As sementes da cultivar Pitiúba são mais tolerantes à salinidade do que as cultivares Canapu e Epace-10.

Termos para indexação: *Vigna unguiculata*, cloreto de sódio, germinação,vigor.

INTRODUCTION

Salinization of agricultural soils is a worldwide concern, especially in irrigated lands, where water is the salt-transporting agent through soil profile. Saline soils present unfavorable conditions for seed germination and plant growth, limiting agricultural production. Irrigation induces an accumulation of salts at soil surface (Klar, 1984), affecting germination, plant stand, plant vegetative development, productivity and, at the worst cases, causes plant death (Silva & Pruski, 1997). In Brazil, salinity generally occurs in alkaline soils of the northeastern semi-arid region (Barbosa, 1996). The climate and soil conditions at the irrigated agriculture region of Juazeiro-Petrolina, are favorable to the soil salinization processes, limiting crop production.

Several studies have been accomplished to elucidate the salinity adaptation mechanisms (Silva et al., 1992). One of the most worldwide spread methods for determination of plant

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tolerance to salts is the germination percentage in salt solutions. The evaluation of germination of salt treated and control seeds are being used as an indicator of the tolerance of some species and cultivars to salinity. As well as germination tests, vigor tests are useful to evaluate the seeds physiological quality during salt stress. These evaluations are important to estimate the performance potential of the seeds at the field in salt stressing environments.

In the areas North and Northeast of Brazil, cowpea is a spread legume representing about 80% of the total production of grains for the human feeding, as a green vegetable or dry beans. This crop is very similar to bean and is developed thoroughly in Africa, India and Brazil, constituting an important protein (23-30%) and carbohydrate (56-68%) source (Bressani, 1993). It is also widely grown elsewhere for different proposes, such as a source of green manure organic matter for poor or depleted soils and fodder crop for livestock.

This work aimed to evaluate seed physiological response of *Vigna unguiculata* (L.) Walp (cowpea) at different salt concentrations.

MATERIAL AND METHODS

The experiment was carried out at Petrolina, Pernambuco State, Brazil. Four replications of 50 seeds of the cultivars 'Epace-10'; 'Canapu' and 'Pitiúba' of cowpea (*Vigna unguiculata* L. Walp), were submitted to germination test in germinator at 25^{0}C, according to a totally randomized design. These seeds germinated in "germitest" papers imbibed in distilled water (H_2O) or in sodium chloride (NaCl) solutions in a proportion of 2,5 times the weight of the paper (Menezes et al., 1993). The NaCl concentrations were 0, 10, 50, 100 e 200mol.m^{-3}, which electrical conductivity (EC, S m^{-1}) was measured with a conductivimeter and the osmotic potential (Ψ_π, MPa) was calculated according to Vijayan (2003).

The evaluations were carried out according to the Rules for Seed Analysis (Brasil, 1992) at the first and second counting, 5 and 8 days for cowpea. The data were accessed for percentage of normal seedlings. After germination evaluations, 10 normal seedlings were oven dried at 65^{0}C, during 72 hours, and weighted, determining the dry matter weight (DW). Fresh matter weight (FW) was also accessed before drying the seedlings (Nakagawa, 1999).

The seedlings growth evaluation was accomplished according to Nakagawa (1999). Ten seeds were distributed along a line drawn longitudinally at 100mm from the upper border of the germination paper pre-imbibed in the H_2O and NaCl solutions mentioned above. Paper rolls were similar to germination test (Brasil, 1992). After 9 days in a 25^{o}C germinator, the hypocotyl length (HL) and root length (RL) were measured. With these data the total seedlings length (TL) and the HL:RL ratio were calculated.

Total proteins content in the seedlings cotyledons were obtained from 3, 5 and 8 days germinated seedlings. The cotyledons were macerated in a 50mol m^{-3} potassium phosphate buffer, pH 7.0 and centrifuged at 5000xg for 10 minutes. The proteins were quantified according to Bradford (1976).

The experimental data were statistically analyzed by variance analysis, for comparison of means by Tukey test (α=0.05).

RESULTS AND DISCUSSION

The seeds were subjected up to 1.874 S m^{-1} and -0.488 MPa (Table 1). In many species these values are too high for water uptake, seed imbibition and germination (Murillo-Amador & Troyo-Diéguez, 2000).

The physiological quality of the different cowpea (*Vigna unguiculata*) cultivars was similar in non-stressed seeds. Although, in treated seeds, the results revealed that the vigor of Canapu and Epace 10 cultivars at the first counting were lower than cv. Pitiúba seeds, for all the NaCl concentrations. This result shows a higher seed tolerance of cv. Pitiúba to salt stress than cvs. Canapu and Epace 10. The germination percentage of the cv. Pitiúba decreased only on the highest NaCl concentration (200mol.m^{-3}), while the germination of the other two cultivars decreased at 100mol.m^{-3}, evidencing the higher tolerance of cv. Pitiúba to salinity (Table 2).

Concerning to the seedlings growth, the cv. Pitiúba showed again to be the most tolerant to the salt stress, accumulating higher fresh matter weight (FW) and dry matter weight (DW) on NaCl 10mol m^{-3} solution.

The cv. Canapu seeds, the most sensitive to salt, FW decreased on 50mol m^{-3} NaCl concentrations and in the other cultivars this occured only on NaCl 100mol.m^{-3} (Table 3).

TABLE 1. NaCl concentration, electrical conductivity (EC) and the osmotic potential (Y_p) of the solutions in wich the seeds germinated. Petrolina, August 2003.

NaCl concentration (mol m^{-3})	Electrical conductivity EC (S m^{-1})	Osmotic Potential -Ψ (MPa)
0	0.00	0.000
10	0.11	-0.024
50	0.45	-0.122
100	1.02	-0.244
200	1.87	-0.488

Cowpea seeds germination was significantly reduced when electrical conductivity in sand culture exceeded 1.2 S m^{-1} (West & Francoise, 1982), decreasing 30% when exposed to 1.56S m^{-1} (Murillo-Amador & Troyo-Diéguez, 2000). Similar results were obtained in our study because germination of Pitiúba, Epace 10 and Canapu seeds decreased, respectively, 6.1, 10.7 and 25% when treated with NaCl 100mol m^{-3}, the equivalent to 1,02S m^{-1}.

All cultivars presented decreased hypocotyls length on NaCl 100mol m^{-3} and decreased root and total length at NaCl 50mol m^{-3}. Seedlings of cvs. Pitiúba and Canapu treated with NaCl 10mol m^{-3} had longer hypocotyl length in relation to control seedlings. This might have occurred due a possible priming effect caused by low NaCl concentration. Priming can improve seed performance, due to its effect in repairing damaged membranes and organelles, resulting in a faster seed emergence and higher seedling growth (McDonald, 1998).

Evaluating cowpea response to salinity, differences were observed among the cultivars only in hypocotyl length (Table 4). When submitted to 100mol m^{-3} NaCl solutions, the seedlings had hypocotyl length decreased in 70.3, 68.1 and 55.6% for cultivars Epace-10, Pitiúba and Canapu, respectively (Table 4).

According to several authors salt stress decreases growth of calli, as well as adult plants and seedlings roots and shoots (Viegas et al., 1999; Camara et al., 2000; Murillo-Amador& Troyo-Diéguez, 2000).

High salt levels, such as 137mol m^{-3} NaCl decreased maize callus relative growth rate (Camara et al.,2000). Salinity inhibits cowpea shoot and root growth, decreasing drastically at EC higher than 0.78S.m^{-1} (Murillo-Amador & Troyo-Diéguez, 2000). In this experiment, all cultivars responded

TABLE 2. First counting and total germination of seed of different cultivars of cowpea, submitted to salt stress. Petrolina, August 2003.

NaCl (mol m^{-3})	FW (mg)			DW (mg)		
	Cultivars			Cultivars		
	Epace 10	Canapu	Pitiúba	Epace 10	Canapu	Pitiúba
0	3645.25 aA*	3434.25 abA	2378.66 bBC	373.50 aA	361.25 aA	227.33 bBC
10	4191.75 aA	3353.25 aA	4130.50 aA	358.50 aA	353.75 aA	392.00 aA
50	3164.00 aA	1987.25 bB	3311.75 aAB	311.25 aA	220.75 aB	313.75 aAB
100	1272.00 aB	751.75 aC	1376.50 aC	177.75 aB	93.75 aC	174.25 aC
	CV (%) 21.00			CV (%) 20.95		

* Same capital letters in the column and lower case letters in the line, do not differ statistically at 5%.

TABLE 3. Fresh matter weight (FW) and dry matter weight (DW) seedlings of different cultivars of cowpea, submitted to salt stress. Petrolina, August 2003.

NaCl (mol m^{-3})	FW (mg)			DW (mg)		
	Cultivars			Cultivars		
	Epace 10	Canapu	Pitiúba	Epace 10	Canapu	Pitiúba
0	3645.25 aA*	3434.25 abA	2378.66 bBC	373.50 aA	361.25 aA	227.33 bBC
10	4191.75 aA	3353.25 aA	4130.50 aA	358.50 aA	353.75 aA	392.00 aA
50	3164.00 aA	1987.25 bB	3311.75 aAB	311.25 aA	220.75 aB	313.75 aAB
100	1272.00 aB	751.75 aC	1376.50 aC	177.75 aB	93.75 aC	174.25 aC
	CV (%) 21.00			CV (%) 20.95		

* Same capital letters in the column and lower case letters in the line, do not differ statistically at 5%.

TABLE 4. Hypocotyl length and root length of cowpea seedlings submitted to salt stress. Petrolina, August 2003.

NaCl (mol m^{-3})	Hypocotyl length (mm)			Root length (mm)		
	Cultivars			Cultivars		
	Pitiúba	Epace 10	Canapu	Pitiúba	Epace 10	Canapu
0	62.5 abB*	67.1 aA	44.9 bB	124.4 aA	142.8 aA	136.9 aA
10	99.9 aA	74.8 bA	73.5 bA	125.3 aA	128.0 aA	135.4 aA
50	49.0 AB	42.6 aB	37.6 aBC	87.4 aB	96.8 aB	9.96 aB
100	19.9 aC	1.9 a C	1.9 aC	41.1 aC	55.8 aC	48.2 aC
	CV (%) 20.68			CV (%) 13.44		

* Same capital letters in the column and lower case letters in the line, do not differ statistically at 5%.

similarly in root and total length. In 100 and 200mol m^{-3} NaCl, which corresponds to 1.02 and 1.87S m^{-1}, growth of salt stressed seedlings decreased (Tables 3 and 4). Canapu was the only cultivar that presented differences in root:hypocotyl ratio, at 10mol m^{-3} NaCl, comparing to controls (Table 5).

Cowpea has been reported to have a good tolerance to heat, drought and salt stress (Rachie & Roberts, 1974; West & Francoise, 1982; Maas, 1986; Maas & Poss, 1989), responding positively to irrigation but will also produce well under dryland conditions (Davis et al., 2003). Drought resistance is one reason that cowpea is such an important crop in northeastern region of Brazil along with, many other underdeveloped parts of the world (Queiroz et al., 2004). Due to the osmotic pressure caused by NaCl, drought stress is intimately related to salt stress. According to Bernstein & Hayward (1958), it is required an osmotic potential (Ψ_π) of -0.4MPa to reduce 50% beans growth. In this experiment the Ψ_π, were 0, -0.024, -0.122, -0.244 and -0.488 MPa for 0, 10, 50, 100 and 200mol m^{-3} NaCl, respectively, thus it is likely that the 100 and 200mol.m^{-3} NaCl concentrations inhibit seed germination and seedlings growth.

Under high water stress (a shift of -1.0 to -2.0 MPa), some plants produce in abundance low-molecular-weight substances that lower the solute potential, such as amino acids and polyamines. The lower solute potential would cause an overall drop in water potential, so that water would still move into the cells and restore turgor (Marvel, 2003). Camara et al. (2000) observed an increase in proline, arginine, γ-amino butyric acid, alanine, glutamine and glutamate in maize calli subjected to NaCl concentrations higher than 100mmol.L^{-1}. In spite of that, Dell´Aquila & Spada (1993) observed that protein synthesis is strongly inhibited by salt stress. Cowpea cotyledons total protein content was observed in three occasions, at radicle protrusion (3 days after sowing, DAS), at first germination counting (5 DAS) and at second germination counting (8 DAS), in which the response presented by the cultivars was quite different (Table 6). The NaCl treatments induced, at 3 DAS, a decrease in total protein content of Canapu cotyledons, on 50mol.m^{-3} NaCl treatment Canapu cotyledons had the lowest protein content. On the other hand, Pitiúba and Epace-10 cotyledons had a little decrease in protein content until 50mol m^{-3} and on 100 mol m^{-3} proteins reached the highest level. The seeds had similar response at 5 and 8 DAS, with increased protein content on 10mol m^{-3} NaCl and a great reduction in higher concentrations, except for Epace-10 which had an increase of protein content at 5 DAS and Pitiúba which protein content was maintained high with 50mol m^{-3} NaCl at 8 DAS. Salinity modulates the production of selected groups of proteins named "salt stress proteins" (Dell´Aquila & Spada, 1993). These results suggest that the pattern of "salt stress proteins" synthesis or protein turnover is different, according with NaCl concentration and with duration of salt stress.

TABLE 5. Root: hypocotyls ratio and total length of cowpea seedings submitted to salt stress. Petrolina, August 2003.

NaCl (mol m^{-3})	Root:hypocotyl ratio			Total length (mm)		
	Cultivar			Cultivar		
	Pitiúba	Epace 10	Canapu	Pitiúba	Epace 10	Canapu
0	2.52 aA*	2.36 aA	3.28 aA	186.9 aB	209.9 aA	181.9 aA
10	1.56 aA	2.08 aA	2.14 aB	225.2 aA	202.9 aA	208.9 aA
50	2.14 aA	2.37 aA	2.77 aAB	136.4 aC	139.4 aB	137.3 aB
100	2.15 aA	2.37 aA	2.77 aAB	61.0 aD	75.8 aC	68.2 aC
	CV (%) 11.50			CV (%) 11.50		

*Same capital letters in the column and lower case letters in the line, do not differ statistically at 5%.

TABLE 6. Protein content (mg g^{-1} of dry matter) in cotyledons of germinating cowpea seeds submitted to salt stress.Petrolina, August 2003.

NaCl (mol m^{-3})	3 days after sowing			5 days after sowing			8 days after sowing		
	Cultivar			Cultivar			Cultivar		
	Pitiúba	Epace 10	Canapu	Pitiúba	Epace 10	Canapu	Pitiúba	Epace 10	Canapu
0	120.74 bB*	112.87 bB	210.97 aA	68.63 abD	74.45 aC	61.96 bD	212.87 cD	67.61 bC	93.86 cB
10	93.72 aC	101.60 aBC	96.46 aC	250.75 aA	79.22 cC	191.79 bA	210.61 aB	180.75 bA	199.87 aA
50	115.10 aB	97.71 BC	36.57 cD	107.71 bC	121.17 aB	99.26 bC	232.21 aA	106.54 bB	109.45 bB
100	154.80 aA	158.08 Aa	129.36 bB	209.68 aB	173.86 bA	171.81 bB	90.24 aC	64.32 bC	93.14 aB
	CV=11.44			CV=12.57			CV=13.95		

* Same capital letters in the column and lower case letters in the line, do not differ statistically at 5%.

Although exists a great amount of literature describing the effects of salinity in adult cowpea plants (Franco et al., 1999; Silva et al., 2003). However, its effects during seed germination in different cultivars still remains unclear. Due to this fact more studies with salt stressed seeds germination are necessary for the complete elucidation its effect on cowpea development.

CONCLUSIONS

Salt concentrations higher than 50mol m^{-3} NaCl affect the germination of cowpea seeds, as well as the cowpea seedlings growth and cotyledons total protein synthesis to all cultivars.

The seeds of cultivar Pitiúba is more tolerant to salinity, than the cultivars Canapu and Epace 10.

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