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Bean seeds under salt stress as a function of nitric oxide

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The objective of this study was to evaluate the bean seeds under salt stress as a function of nitric oxide (NO). Four bean genotypes (*Phaseolus vulgaris* L.) seeds G1 = peanut; G2 = white *carioca*; G3 = red *carioca* and G4 = black that were previously immersed in four sildenafil citrate concentrations: 0; 25; 50 and 75 mg L^{-1} for 120 min, and placed to germinate under induced stress with NaCl at -0.8 MPa, on a germitest paper roll. The design was completely randomized, with four replicates per treatment and for the effect of sildenafil citrate concentrations, the data were submitted to regression analysis. Salinity stress with sodium chloride at -0.8 MPa reduces bean genotypes germination and seed vigor. Sildenafil citrate at a concentration of 50 mg L^{-1} reduced the effect of salinity stress on bean genotypes, and G1 and G3 showed a greater response under salinity stress with sodium chloride at -0.8 MPa.

Keywords: *Phaseolus vulgaris* L., Sodium chloride, Salinity, Phytochemical, Sildenafil Citrate, Germination

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Bean (*Phaseolus vulgaris* L.) is a legume of great socioeconomic importance, widely distributed throughout the world, and is widely consumed by Brazilian population, standing out as an important source of proteins, vitamins and iron[1]. Considering the three harvests in 2016, the national production of beans suffered a decrease of 6.1 % compared to the harvest of 2015, totaling 2,925.7 thousand tons. However, it is estimated around 3.4 million tons for the grain harvest 2016/2017, with estimated production for the first harvest of 1,596,692 tons; for the second harvest, 1,227,438 tons, and in the third harvest, 462,009 tons, with estimated production of 512.1 thousand tons of common black bean[2]. The planted area had an increase of 16.7 % and the average yield increased by 9.8 % compared to 2016[3].

The common bean comes from the American continent, belonging to the family Fabaceae, genus *Phaseolus*, in which there are more than 60 species, with different color, sizes and grain shapes. Only five species are commercialized, especially the species *Phaseolus vulgaris* L., commonly known as common bean, which is cultivated by 107 countries worldwide, being the most cultivated: *P. vulgaris* L., *P. lunatus* L., *P. coccineus* L., *P. acutifolius* A. Gray *var. latifolius* Freemane and *P. polyanthus* Greenman[4].

The water and salinity stresses limited the production of several crops, causing morphological, physiological and biochemical changes in the plants, especially when exposed to high concentrations, reducing their development, growth, osmotic potential, productivity and accumulation of ions in plant tissues, which causes toxicity and important nutritional imbalances[5].

The decreased germination in bean seeds, when submitted to water stress, is caused by reducing the volume of water necessary to activate the metabolic processes of the cells[6]. Salinity stress decreases seed germination, due to the accumulation of Na^{+}, which transforms the chemical balance inside the cells, due to the great supply of sodium, and consequently reduces the cell division and the development of the embryo[7].

Therefore, studies have been carried out to verify the occurrence of osmotic stresses with the use of mannitol, which is enough to cause decreased

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germination in water deficit in bean seeds[8]. In addition, the use of sodium chloride to identify salinity stress, observed that the absorption of Na^+ and Cl^- ions are toxic to the seeds, and caused water to enter into the cells due to the reduction in the osmotic potential[9].

The objective of this study was to evaluate the physiological quality of bean seeds treated with sildenafil citrate and submitted to salinity stress.

Materials and methods

Plant material

The experiment was carried out in the Seed Analysis Laboratory (LAS) of the Center of Agricultural Sciences and Engineering (CCAE) of UFES, *Alegre*-ES, using four bean genotypes seeds (*Phaseolus vulgaris* L.) G1 = peanuts; G2 = white *carioca*; G3 = red *carioca* and G4 = black. Seeds of bean genotypes from the region's farmers were planted at the BarroBranco site in Rive, Alegre-ES district and the seeds were harvested in July 2017.

Treatment with sildenafil citrate

The seeds were immersed in four sildenafil citrate concentrations: 0, 25, 50 and 75 mg L^{-1} for 120 min, afterwards they were washed. Germination test was carried out using a germitest paper soaked with distilled water and NaCl solution with potential of -0.8 MPa, equivalent to three times the dry paper mass, to simulate the absence or presence of salinity stress.

The rolls were kept in BOD Incubator Seed Germinator Oven at a constant temperature of 25 ºC, constant light, for the germination experiment. The treatments were composed of the following combinations: [genotypes (4) x sildenafil citrate concentration (4)] and, in the absence and presence of salinity stress (NaCl -0.8 MPa).

Physiological quality of the seed

The germination was evaluated daily for nine days, and the germinated seeds were considered to have a protrusion of the primary root with length ≥ two mm. The data obtained from the daily counts were used in the calculation of the germination speed index (GIS), according to Maguire (1962)[10]. In addition, shoot length was evaluated nine days after sowing, using a millimeter rule, by measuring the length between the collar and the apex of the last leaf of each sample plant and the results were expressed in cm $plant^{-1}$. The root length was obtained by measuring from the collar of the plant to the tip of the largest root and the results were expressed in cm $plant^{-1}$. The root and seedling fresh and dry mass of the aerial part was determined nine days after sowing, on an analytical balance (0.0001 g). After obtaining the fresh mass, the seedlings were packed in a Kraft paper bags, kept in a convection oven at 72 ºC for 72 h. The samples were stored in desiccator with silica and then weighed, and the results were expressed in mg $seedling^{-1}$[11].

Statistical analysis

The design was completely randomized, with four replications per treatment. The data were submitted to the residue normality test and the analysis of variance. For the effect of sildenafil citrate concentrations, the data were submitted to regression analysis and, for the adjustment of the quadratic equations ($\hat{Y}$ =), the significance of betas ($p \leq 0.05$) and R 2 > 0.50. For all analyzes the software R was used using the Exp Des package[12].

Results and discussion

Saline stress with sodium chloride

There was a significant difference between the genotypes and salinity stress with sodium chloride (Table 1). Bean seeds treated with sodium chloride at

Table 1 — Germination (G), germination speed index (GSI), shoot length (SL) and root length (RL) of bean genotypes in the presence and absence of sodium chloride (-0.8 MPa)[(1)]

Genotypes	G (%)		GSI		SL (cm)		RL (cm)	
	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl
G1	98aA	97aA	15.31 cA	11.36 aB	12.87 cA	3.28 bB	12.78 aA	4.68 cB
G2	94bA	91bB	17.46 abA	12.12 aB	15.18 bA	6.35 aB	12.97 aA	6.13 bB
G3	99aA	98aA	16.00 bcA	12.44 aB	17.32 aA	7.63 aB	13.61 aA	7.70 aB
G4	94bA	87cB	18.50 aA	11.71 aB	12.95 cA	6.53 aB	11.61 bA	5.76 bB
CV (%)	1.69		6.47		6.49		4.65	

[(1)]Means followed by the same letter, lower case in the column and upper case in the row do not differ by Tukey test, at a 5 % probability level.

G1: peanut; G2: white carioca; G3: red carioca; G4: black; - NaCl: absence of sodium chloride and + NaCl: presence of sodium chloride (-0.8 MPa).

-0.8 MPa presented lower values for germination (G), germination speed index (GIS), shoot length (SL) and root length (RL).

A lower germination percentage and root length with increasing salinity were found in the bean crop by Habtamu *et al.*[13], which corroborates with the results found in this study and in other studies[14-18,7]. Low water potential reduces seed germination due to a decrease in water availability required to activate and maintain the seed metabolism[19], and this decreased water potential, associated with the toxic effect of the salts, with initial interference in the process of water uptake by seeds, influences germination, seedlings vigor and normal development of plants[20].

The genotypes of peanut (G1) and red *carioca* (G3) presented higher germination values in the absence (98 and 99 %, respectively) and presence (97 and 98 %, respectively) of sodium chloride at -0.8 MPa in relation to the other genotypes. For the germination speed index, there was no significant difference between the genotypes in the absence and presence of salinity stress. G3 presented higher values of shoot length in the absence of sodium chloride (17.32 cm) and higher values of root length in the presence of salinity stress (7.70 cm) in relation to the other genotypes.

In the present study, it was observed a height reduction due to the increased salinity in different crops, such as bell pepper[21], coriander[22], bean[13], fodder sorghum[23] and cowpea[24], similar to the results observed in this study. The decrease in shoot length may be due to salinity stress since there is a change in the hormonal balance caused by the release of abscisic acid with consequent inhibition of auxin, the main hormone responsible for plant growth[25].

The growth and development of plants are affected by salinity and the effects depend on the plant species[16] and also the plant part that was affected. However, the aerial part is always the most affected; reducing root and fresh and dry mass of the aerial part of the common bean[26]. Sensitivity to different stress levels may vary from genotype to genotype and how long they were exposed to the saline[27], and stress adaptation may vary between species and in phenological stages may vary in the same genotype[28].

There was a significant difference between bean genotypes and the presence and absence of NaCl (-0.8 MPa) for all analyzed variables, except for root dry matter (RDM) (Table 2).

Considering shoot fresh mass (SFS) in the absence of NaCl, the peanut genotype (G1) presented the highest mass (1637.99 mg) when compared to the others. However, in the presence of NaCl, there was no difference between the genotypes for FSS. All genotypes showed higher SFS in the absence of NaCl, when values were compared in the presence of NaCl. This behavior is due to an unbalanced nutrient absorption under salinity stress condition with damages to membrane systems and disturbances in cell physiology, due to the loss of protoplasmic turgescence[29].

While in the root system, the peanut genotype (G1) presented a higher root dry mass (RDM) (396.83 mg) when compared to the other genotypes, in the absence of NaCl. In the NaCl treatment, the peanut genotype presented similar RFM (246.82 mg) between the genotypes white *carioca* (G2) (216.30 mg) and red *carioca* (G3) (280.45 mg), and they differed from each other. The black genotype (G4) had the lowest RFM value (174.44 mg). All genotypes showed higher RFM in the absence of NaCl, when values were compared in the presence of NaCl (Table 2). The presence of NaCl reduced RFM by reducing the water conductivity of common bean roots and culminating with lower tissue elongation and interference in the carbohydrate synthesis process

Table 2 — Shoot fresh mass (SFM), root fresh mass (RFM) and shoot drymass (SDM) of bean genotypes in presence and absence of sodium chloride (-0.8 MPa)[(1)]

Genotypes	SFM (mg)		RFM (mg)		SDM (mg)	
	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl
G1	1637.99 aA	693.21 aB	396.83 aA	246.82 abB	162.93 aA	150.47 aB
G2	1059.56 bA	702.97aB	328.47 bA	216.30 bB	133.25 bA	110.09 bB
G3	1080.26 bA	673.13 aB	340.22 bA	280.45 aB	119.97 cA	90.26 cB
G4	1005.57 bA	702.69 aB	257.68 cA	174.44 cB	142.26 abA	117.99 bB
CV (%)	8.39		8.30		4.48	

[(1)]Means followed by the same letter, lower case in the column and upper case in the row do not differ by Tukey test, at a 5 % probability level.

G1: peanut; G2: white carioca; G3: red carioca; G4: black; - NaCl: absence of sodium chloride and + NaCl: presence of sodium chloride (-0.8 MPa).

caused by water and salinity stress[30]. In the treatment with absence of NaCl for SDM, the peanut genotype (G1) presented the highest value (162.93 mg) when compared to the other genotypes. In the presence of NaCl, the peanut genotype (G1) presented the highest SDM values (150.47 mg), corroborating with the results obtained by[31] in seedlings from common bean seeds, cultivar *Pérola*.

Saline stress with sodium chloride submitted to sildenafil citrate

There was a significant interaction between genotypes and sildenafil citrate concentrations in the presence of salinity stress with sodium chloride at -0.8 MPa (Fig. 1).

According to the increase of the sildenafil citrate concentrations, there was a decrease in the germination and germination rate up to the concentration of 50 mg L^{-1}. The concentration of 50 mg L^{-1} had an increase in the germination and germination speed index, in which sildenafil citrate reduces the effect of salinity stress with sodium chloride, increasing the germination percentage and common bean genotypes vigor (Fig. 1).

Sildenafil Citrate, an active chemical in Viagra®, a drug used to treat male erection is a nitric oxide donor[32]. Nitric oxide is a signaling molecule in plants which regulates plant growth and development, in the defense against pathogens and responses to abiotic stress[33].

The different sildenafil citrate concentrations for G1, G2 and G3 were not significant for germination. The sildenafil citrate concentration of 0 mg L^{-1}, G4 presented 95 % and at 85 mg L^{-1} presented 85 %, with the increase of sildenafil citrate concentration of 75 mg L^{-1} presented 88 % germination (Fig. 1A).

G4 that was subjected to salinity stress with sodium chloride at -0.8 MPa to 0 mg L^{-1} of sildenafil citrate presented germination speed index of 13.98, at 25 mg L^{-1} presented 9.68 and with increased sildenafil citrate concentration of 75 mg L^{-1} showed 12.04. The different sildenafil citrate concentrations for G1 and G3 were not significant germination rate (Fig. 1B).

Salinity stress caused reduction in germination, a significant increase in the superoxide dismutase, catalase and ascorbate peroxidase activity in cucumber seeds found by Fan *et al.* (2013)[34] and in basil seeds by Saeidnejad *et al.* (2013)[26] in which nitric oxide induced salt tolerance avoiding oxidative damage to the seeds. Silva *et al.* (2015)[35] in *Senna macranthera* seeds studied the effect of sodium nitroprusside in providing nitric oxide and showed that it has the potential to promote the recovery of seed germination under salinity stress with sodium chloride.

The use of sildenafil citrate is a viable alternative as a growth regulator, promoting tolerance in plants when induce to salinity stress[36,37].

There was a significant interaction between genotypes and sildenafil citrate concentrations in the presence of salinity stress with sodium chloride at -0.8 MPa (Fig. 2).

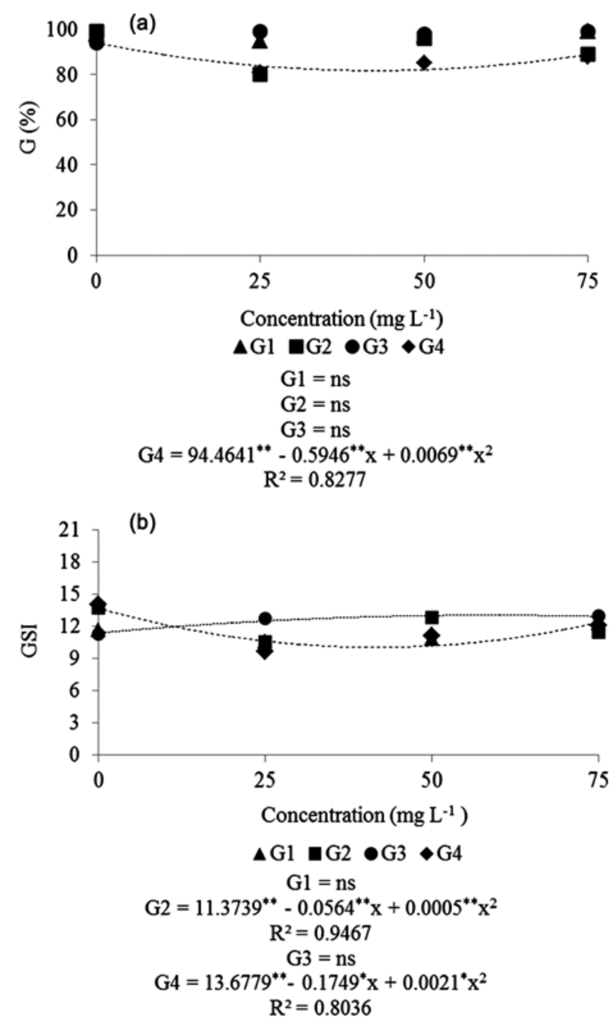

Fig. 1 — Germination (G – A) and germination speed index (GSI – B) of bean genotypes (G1: peanut; G2: white carioca; G3: red carioca and G4: black) at different concentrations of sildenafil citrate in the presence of NaCl (-0.8 MPa).
**, *and ^{ns}: significant at 1 %, 5 % and not significant, respectively. R^2 = coefficient of determination of the regression.

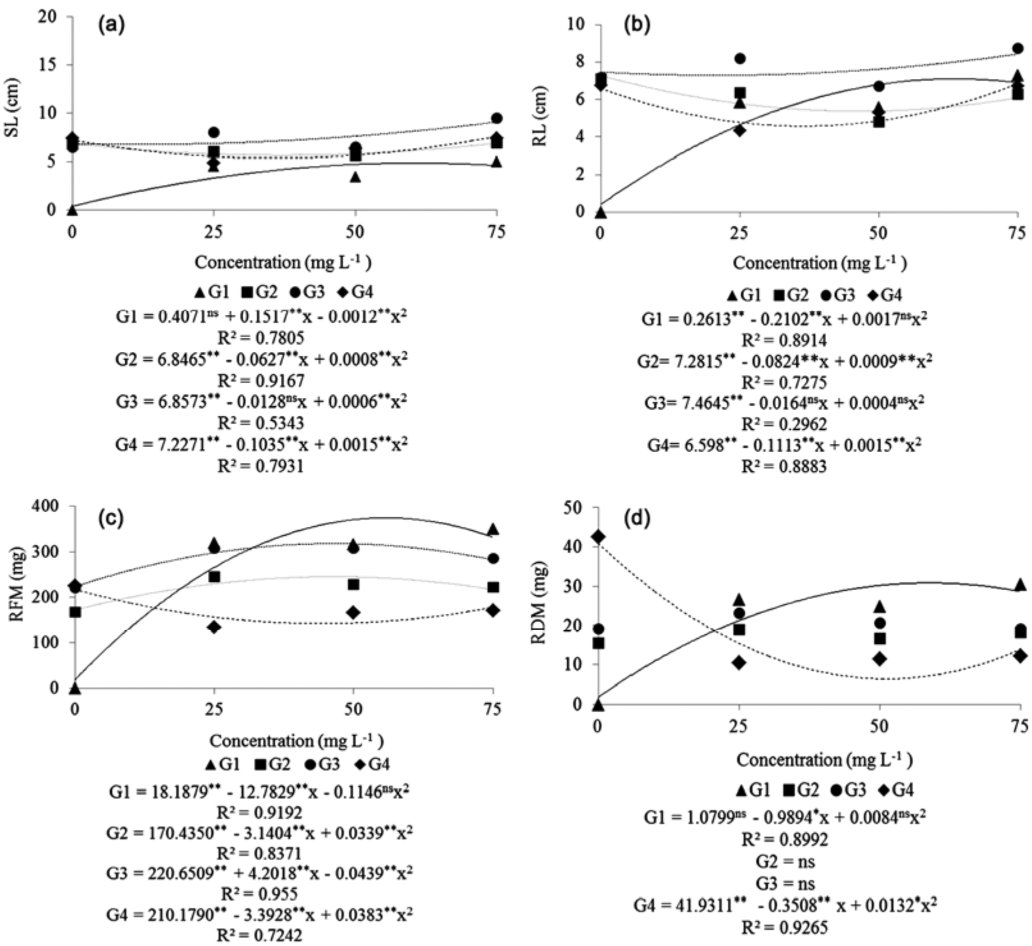

Fig. 2 — Shoot length (SL - A), root length (RL - B), root fresh mass (RFM - C) and root dry mass (RDM - D) of bean genotypes (G1: peanut; G2: white carioca; G3: red carioca and G4: black) at different concentrations of sildenafil citrate in the presence of NaCl (-0.8 MPa). **, *and^{ns}: significant at 1 %, 5 % and not significant, respectively. R^2 = coefficient of determination of the regression.

According to the increase in the sildenafil citrate concentrations there was a reduction in shoot length, root, fresh and dry mass of bean genotypes under salinity stress to 50 mg L^{-1} concentration. At the concentration of 50 mg L^{-1}, there is an increase in the response to root length, root, fresh and dry root weight variables in which sildenafil citrate reduces the effect of salinity stress, increasing the bean genotypes vigor (Fig. 2).

The G3 subjected to salinity stress with sodium chloride at -0.8 MPa in different sildenafil citrate concentrations presented higher values of shoot and root length compared to the other genotypes. Sildenafil citrate G3 at 0 mg L^{-1} presented shoot length of 6.47 cm and with the increase of the sildenafil citrate concentration 75 mg L^{-1} presented 9.50 cm (Fig. 2A). G3 with sildenafil citrate at 0 mg L^{-1} presented root length of a 7.16 cm and with an increase in the sildenafil citrate concentration75 mg L^{-1} presented 8.73 cm (Fig. 2B). Corroborating with the results obtained by Yakovishin *et al.* (2014)[37], in *Avena sativa* L. seeds, in which there was an increase in the aerial part and the root length with the application of sildenafil citrate.

The G1 subjected to salinity stress with sodium chloride at -0.8 MPa in different sildenafil citrate concentrations presented higher values of shoot length and root length compared to the other genotypes. The G1 subjected to salinity stress with sodium chloride at -0.8 MPa at 0 mg L^{-1} of sildenafil citrate presented 0 mg of fresh root mass root fresh mass and with the increase of the sildenafil citrate concentration75 mg L^{-1} presented 350.37 mg (Fig. 2C).

For the variable root dry mass, it was observed that the G1 subjected to salinity stress with sodium chloride at -0.8 MPa at 0 mg L^{-1} of sildenafil citrate presented 0 mg and with the increase of the sildenafil citrate concentration75 mg L^{-1} showed 97.33 mg. The different sildenafil citrate concentrations for G2 and G3 were not significant for root dry mass (Fig. 2D).

Traditional significance of study

The seeds exhibit sensitivity to water stress and saline, which reduce the osmotic potential and affect water uptake by seeds, which determines a reduction in physiological and biochemical processes, and in the arid and semi-arid areas for excess salts, which cause the water deficit and affect agricultural production.

Conclusion

The salt stress induced with sodium chloride -0.8 MPa reduces the physiological quality of seeds of bean genotypes. The use of nitric oxide donor Sildenafil Citrate -0.8 MPa reduces the saline stress in genotypes of bean seeds.

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References

1 Mapa (Ministry of Agriculture and Supply). Bean. Available from: http://www.agricultura.gov.br/vegetal/culturas/feijao. Accessed 02 November 2017, 2017.

2 Conab (National Supply Company). Follow-up of the Brazilian grain crop. Available from: www.conab.gov.br /OlalaCMS/uploads/arquivos/17_07_12_11_17_01_boletim_g raos_julho_2017.pdf>. Accessed 16 July 2017, 2017.

3 Ibge (Brazilian Institute of Geography and Statistics), *Systematic Survey of Agricultural Production*, Vol 30, (Publications and Information Directorate, Rio de Janeiro), 2017, 1-83.

4 Vieira C, Paula Júnior TJ & Borém A, Bean, (Viçosa, MG), 2006, 600.

5 Silva FLB, Lacerda CFDE, Neves ALR, Sousa GG, Sousa CHC, *et al.,* Irrigation with saline waters and use of bovine biofertilizer in the gas exchanges and yield of bean-rope, *Irrigation*, 18 (2) (2013) 304-317.

6 Santos LA, Soratto RP, Fernandes AM & Gonsales JR, Growth, physiological indexes and productivity of common bean cultivars under different levels of fertilization, *Ceres*, 62 (1) (2015) 107-116.

7 Soares M, Santos Júnior HC, Simões MG, Pazzin D & Silva LJ, Hydric and saline stress in soybean seeds classified in different sizes, *Pesqui Agropecu Trop,* 45 (4) (2015) 370-378.

8 Machado Neto N, Custódio CC, Costa PR & DonáFL, Water deficiency induced by different osmotic agents in the germination and vigor of bean seeds, *Rev Bras Sementes,* 28 (1) (2006) 142-148.

9 Michels AF, Souza CA de, Coelho CMM & Zilio M, Physiological quality of *Creole* bean seeds produced in the west and plateau of Santa Catarina, *Rev Cienc Agron,* 45 (3) (2014) 620-632.

10 Maguire JB, Speed of germination-aid in selection and evaluation for seed ling emergence vigor, *Crop Sci*, 2 (2) (1962) 176-177.

11 Brazil (Ministry of Agriculture and Agrarian Reform), National Secretariat of Agricultural and Live stock Defense. *Rules for Seed Analysis*. National Department of Plant Protection. Coordination of Plant Laboratory, Brasília, DF, 2009, 399.

12 R Core Team. *A language and environment for statistical computing.* R Foundation for Statistical Computing. Vienna, Austria, Available from: http://www.R-project.org/, Accessed 07 July 2017, 2017.

13 Habtamu A, Estifanos E & Endale M, Seed germination and early seedling growth of haricot bean (*Phaseolus vulgaris* L.) cultivars as influenced by salinity stress, *Int J Agric Sci,* 4 (2) (2014) 125-130.

14 Kaiser IS, Machado LC, Lopes JC & Mengarda LHG, Effect of nitric oxide releasers on the physiological quality of cabbage seeds under salinity, *Ceres,* 63 (1) (2016) 39-45.

15 Silva MLM, Alves EU, Bruno RLA, Santos-Moura SS & Santos Neto APS, Germination of Chorisiaglaziovii O. Kuntze seeds subjected to water stress at different temperatures, *Sci For***,** 26 (3) (2016) 999-1007.

16 Martins CC, Pereira MRR & Lopes MTG, Germination of *Eucalyptus* seeds underwater and saline stress, *Biosci J,* 30 (3) (2014) 318-329.

17 Pereira MRR, Martins CC, Martins D & Silva RJN, Water stress induced by PEG and NaCl solutions in the germination of glabrous and fedegoso seeds, *Biosci J*, 30 (3) (2014) 687-696.

18 Guimarães IP, Oliveira FN, Vieira FER & Torres SB, Effect to irrigation water salinity on emergence and initial growth of mulungu seedlings, *Rev Bras Cienc Agrar,* 8 (1) (2013) 137-142.

19 Bewley JD, Bradford K, Hilhorst H & Nonogaki H, *Seeds: Physiology of development, germination and dormancy*, 3rd, (New York: Springer), 2013.

20 Ghaderi-Far F, Gherekhloo J & Alimagham M, Influence of environmental factors on seed germination and seedling emergence of yellow sweet clover (*Melilotus officinalis*), *Planta Daninha*, 28 (3) (2010) 436-469.

21 Nascimento IB, Medeiros JF & Alves SSV, Initial development of pepper culture influenced by salinity of irrigation water in two types of soil, *Agrop Cient no Semi-Árido*, 11 (1) (2015) 37-43.

22 Sales MAL, Moreira FJC, Eloi WM, Ribeiro AA, Sales FAL, *et al.*, Germination and initial growth of coriander in substrate irrigated with saline water, *Braz J Biosyst Eng*, 9 (3) (2015) 221-227.

23 Coelho DS, Simões WL & Mendes MAS, Germination and initial growth of forage sorghum varieties submitted to saline stress, *Rev Bras EngAgr Amb,*18 (1) (2014) 25-30.

24 Coelho JBM, Barros MF & Neto EB, Water behavior and growth of *Vigna* beans grown on salinized soils, *Rev Bras Eng Agr Amb,* 17 (4) (2013) 379–385.

25 Taiz L & Zeiger E, *Physiology and plant development*, 6^{rd}, (Porto Alegre: Artmed), 2017, 888.

26 Saeidnejad AH, Pasandi-Pour A, Pakgohar N & Farahbakhsh H, Effects of exogenous nitric oxide on germination and physiological properties of basil under salinity stress, *J Med Plants By-products*, 1 (2013) 103-113.

27 Deuner C, Maia MDS, Deuner S, Almeida ADS & Meneghello GE, Viability and antioxidant activity of seeds of kidney beangeno types submitted to saline stress, *Rev Bras Sementes,* 33 (4) (2011) 711-720.

28 Morales MA, Olmos E, Torrecillas A & Alarcon JJ, Differences in water relations, leaf ion accumulation and excretion rates between cultivated and wild species of *Limonium* sp. grown in conditions of saline stress, *Flora*, 196 (5) (2001) 345-352.

29 Bayuelo-Jimenez JS, Jasso-Plata N & Ochoa I, Growth and physiological responses of *Phaseolus* species to salinity stress, *Int J Agron*, 2012 (2012) 1-13.

30 Coelho DLM, Agostini EATD, Guaberto LM, MACHADO-NETO NB & Custódio CC, Water stress with different osmotic in bean seeds and differential expression of proteins during germination, *Acta Sci-Agron*, 32 (3) (2010) 491-499.

31 Dalchiavon FC, Neves G & Haga KI, Effect of saline stress on seeds of *Phaseolus vulgaris*, *Rev Cienc Agrar*, 39 (3) (2016) 404-412.

32 Siegel-Itzkovich J, Viagra makes flowers stand up straight., *Brit Med J*, 319 (7205) (1999) 274.

33 Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, *et al.,* Nitric oxide (NO) and phytohormonescros stalk during early plant development, *J Exp Bot*, 66 (10) (2015) 2857-2868.

34 Fan HF, Du CX, Ding L & Xu YL, Effects of nitric oxide on the germination of cucumber seeds and antioxidant enzymes under salinity stress, *Acta Physiol Plant*, 35 (9) (2013) 2707-2719.

35 Silva ALD, Dias DCFDS, Ribeiro DM & Silva LJD, Effect of sodium nitro prusside (SNP) on the germination of *Senna macranthera* seeds (DC. Ex Collad.) HS Irwin & Bane by under salt stress, *J Seed Sci*, 37 (4) (2015) 236-243.

36 Zanotti RF, Lopes JC, Motta LB, Freitas AR & Mengarda LHG, Tolerance induction to saline stress in papaya seeds treated with potassium nitrate and sildenafil citrate, *Semin-Cienc Agrar,* 34 (6) (2013) 3669-3674.

37 Yakovishin LA, Lekar AV, Vetrova EV, Borisenko NI, Borisenko SN, *et al*., Molecular Complexes of Ivy and Licorice Saponins with Sildenafil Citrate (Viagra) and Their Biological Activity, *Russ J Bioorg Chem,* 40 (7) (2014) 737–741