

RESEARCH ARTICLE

Germination and seed vigor of *Phaseolus vulgaris* submitted to treatments whit aluminum sulfate

Josimar Aleixo da Silva¹, Allan Rocha de Freitas², Rodrigo Sobreira Alexandre³, Paula Aparecida Muniz de Lima¹, Simone de Oliveira Lopes⁴, Julcinara Oliveira Baptista¹, Tamyris de Mello³, José Carlos Lopes¹

¹Department of Agronomy, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Alegre, ES 29500-000, Brazil, ²Department of Agronomy, Future College (Faculdade do Futuro), Manhuaçu, MG 36900-000, Brazil, ³Department of Forest Sciences, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Jerônimo Monteiro, ES 29550-000, Brazil, ⁴São Carlos Metropolitan College (Faculdade Metropolitana São Carlos), Bom Jesus do Itabapoana, RJ 28360-000, Brazil.

ABSTRACT

The varieties creole are traditionally grown plant, adapted in places where the crops are developed and present in seeds banks of many famers. Thus the objective of this study was to investigate the performance of the germination process and possible toxic effects. Were used seeds of creole cultivars of beans (*Phaseolus vulgaris* L.), using different concentrations of aluminum. We used a randomized completely design with four repetitions of 25 seeds and the treatments were distributed in 4 x 5 factorial scheme, consisting of four bean cultivars (Carioca, Butter, Black, Red) and five concentrations (0.0, 2.5, 5.0, 7.5, and 10.0 mg L⁻¹) of aluminum sulfate. The characteristics analyzed were percentage of germination, percentage of abnormal seedlings, germination speed index, root length, length of the base of the seedling to the hypocotyl, hypocotyl length of seedlings to the epicotyl, root dry mass and shoot dry mass. The variables germination percentage and germination speed index were not affected by the toxic effect of aluminum. The variables percentage of abnormal seedlings, root length and shoot showed a significant reduction with the increase in aluminum sulfate concentrations, thus showing a greater correlation between them.

Keywords: Aluminum; Fabaceae; Physiological quality; Toxicity

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) stands out among grain legumes due to its importance in human consumption attributed to its high nutritional value as a source of vegetable protein, B-complex vitamins, and mineral salts such as iron, calcium, and phosphorus. It is a nutrient-demanding crop due to its small and shallow root system and short cycle, allowing planting in up to three moments during the growing season. Bean production for the 2019/20 crop year is estimated at 3.16 million tons, 4.6% above the 2018/19 production (CONAB, 2020). It is a widely cultivated species worldwide, standing out among economically relevant crops (Pitura and Arntfield, 2019).

From 60 to 80% of the bean produced in Brazil is of the carioca type, although the black, brindle, jalo, red and cowpea types are also grown, suggesting the need for continuous research investments aiming at the development

of new pest-resistant varieties, the tropicalization (adaptation) of varieties of interest for importing countries, and the stability of final prices for the consumer in the internal market, whose per capita consumption ranges from 16 to 17 kg (Brasil, 2018). However, there is a great preference for landrace bean seeds, which is mostly attributed to characteristics such as adaptability, flavor, quality of traditional varieties, and low production costs, constituting a source of starch (41,5%) some vitamins and minerals rich in proteins (34%), carbohydrates, and micronutrients that are valuable in developing countries, being traditionally and widely consumed by the Brazilian population (Campos-Vega et al., 2013; Nemli et al., 2017; Kumar and Pandey, 2020).

The varieties creole may be defined as traditional varieties of cultivated plant, adapted to the locations and cultures where they were developed, present in seeds banks of many agriculturists, mainly in developing countries, because

*Corresponding author:

José Carlos Lopes, Department of Agronomy, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Alegre, ES 29500-000, Brazil. E-mail: jcufes@bol.com.br

Received: 12 July 2023; Accepted: 20 October 2023

they are like a warranty of planting in the next year. The adaptability granted to the traditional varieties is manifested with higher stability and safety in the yields of subsistence farmers, and, therefore, they are their favorite varieties (Coimbra et al., 2010), mainly because root anti-oxidant mechanisms are effective in preventing Al toxicity in plants (Reis et al., 2018).

Germination of the seeds, regardless of the species, is directly influenced by the environmental conditions (Ymashita and Guimarães, 2011). The knowledge of the performance of the seeds' germination process, especially for creole cultivars, under adverse conditions such as the presence of aluminum, is important and highly necessary. The use of nanoparticles of zinc and aluminum oxides has a negative effect on germination, and morphological and genetic characters in fenugreek seeds, with its negative effect more accentuated with the increase in concentrations, in which it was observed that 500 and 1000 mg L⁻¹ of suspensions of zinc and aluminum nanoparticles causes inhibition in the germination of *Trigonella foenum-graecum* seeds (Osman et al., 2020).

Aluminum stress negatively affected seed germination and seedling vigor of *Arctium lappa* L. (Silverio et al., 2021). Machado et al. (2015), when evaluating the germination of *Jatropha curcas* L. seeds exposed to five concentrations of Al, found that the element reduced germination by up to 90%, in addition to reducing 76 and 83% of the length and dry matter of the roots of the seedlings., respectively. In the literature, authors observed that some species also had root growth inhibition in response to Al toxicity, as in *Phaseolus vulgaris* L. (Domingues et al., 2013), *Trifolium pretense* L. (Scheffer-Basso and Prior, 2015) and *Lactuca sativa* L. (Silva and Matos, 2016).

The contamination of water and soils with toxic elements consists of a great environmental problem based on human and animal health. Among the elements toxic in the soil aluminum (Al) stands out, which, when in excess, can cause several different damage to plants decreasing their productivity (Freitas et al., 2012; Chen et al., 2019; Palansooriya et al., 2020).

In acidic soils, Al is solubilized in the soil solution, transforming it into its form trivalent cation (Al³⁺), becoming available to the plant and generating toxicity (Kopittke et al., 2015). Stress by Al is especially characterized by causing growth inhibition of the root, as a consequence of the alteration of different mechanisms and cellular structures, resulting in decreased water and nutrient uptake and plant growth (Eekhout et al., 2017; Reis et al., 2018). This one element affects the synthesis of DNA and the regulation of proteins that control the cell cycle. The Al

toxicity causes disruption of reactive oxygen/Ca²⁺ species homeostasis and Ca²⁺ mediated signaling, considered key events in inducing DNA damage or cell death in plants (Achary et al., 2013). A nature of the beneficial effect of Al on plant growth is unclear, but there is evidence of which is an indirect effect, most often related to competition with other mineral elements present in toxic amounts, mainly copper, zinc and phosphorus (Furlani, 2012).

The objective was to study the physiological quality of seeds of four bean cultivars (Carioca, Butter, Black and Red) submitted to different aluminum cradles (0.0, 2.5, 5.0, 7.5 and 10.0 mg L⁻¹) and their potential phytotoxic effect.

MATERIAL AND METHODS

Plant material

The experiment was conducted at the Laboratory of Seed Analysis of the Center of Agricultural and engineering Sciences in the Federal University of Espírito Santo, located in Alegre-ES. Seeds from for creole cultivars of common beans, in the agricultural year were used, from agriculturists from the city of Castelo located in the south region of Espírito Santo, at latitude of 20°36'13" S and longitude 41°11'05" W. The seeds were purchased and stored in plastic bags, in a cold chamber, at 10 °C, for 30 days, until the moment of analysis.

Seed treatment with aluminum sulphate

The fully randomized design was used, with four repetitions of 25 seeds. The treatments were distributed in the 4 x 5 factorial scheme, composed by four cultivars of beans (Carioca, Butter, Black and Red) and five concentrations (0.0, 2.5, 5.0, 7.5 and 10.0 mg L⁻¹) of aluminum sulfate [Al₂(SO₄)₃·18 H₂O]. The solutions used were obtained by dissolving the aluminum sulfate in distilled water.

Analysis of the physiological quality of seeds

The humidity and the weight of thousand seeds of each batch of cultivar according to Brasil (2009) were provided. The germination percentage (G) was assessed, conducted in germitest paper rolls, damp in the proportion of 2.5 times their dry mass, with solutions containing the different concentrations of aluminum. The paper rolls were placed in vertical position inside the plastic jars containing the proper aluminum concentrations with 125 mL amounts, in order to maintain the base of the rolls always damp. Subsequently, the plastic jars containing the rolls with the seeds were placed in BOD (biochemical oxygen demand) germinator with temperature regulated for 25 °C, with reading being performed in the 5th and 9th days after seeding, calculating the normal and abnormal seedlings (AS) (Fig. 1). The results were expressed in percentages according to the Rules for Seeds Analysis (Brasil, 2009).



Fig 1. Plastic jars containing the rolls with the seeds placed in BOD (biochemical oxygen demand).

Concomitantly with the germination tests, daily readings were performed counting the germinated seeds, determining the germination speed index (GSI) (Maguire, 1962).

And, after that period, ten normal seedlings were randomly removed for the measurements of: shoot length (SL) and root (RL), with the help of a millimeter rule and expressed in cm-m change; dry mass of aerial part (ADM) and root (RDM), in which the parts were placed in bags of the Kluft type and placed in a forced circulation oven at 72 °C for 72 hours, after the samples were removed and weighed on an analytical balance with precision 0.0001 g, and the result expressed in grams.

Statistical analysis

The variance analysis was performed with the help of the application program R (R Core Team, 2018). The data were submitted to the Shapiro-Wilk normality test and for characteristics, abnormal seedlings percentage, AS; root length, RL; length of the hypocotyl of the seedling until the epicotyl, LH; root's dry mass, RDM and aerial part dry mass, ADM were transformed into $(x+0,5)^{1/2}$. The averages were compared by the Scott-Knott test with 5% probability level using the computing program application GENES (Cruz, 2016). In order to detect the influence of aluminum in RL, a cubic regression of the RL was performed in different concentrations of aluminum sulfate.

In order to complement the statistical analysis, correlations (Cruz, 2016) and grouping (R Core Team, 2018) analyses were performed. A1 (aluminum concentrations) variable correlations were performed with G, AS, GSI, RL, LH, LE, RDM and ADM, in addition to the correlation between the analyzed variables, not taking into account A1. The treatments were grouped by the most distant neighbor method and the differences expressed by the generalized distance of Mahalanobis, and a cophenetic correlation was performed in order to verify the grouping's consistency.

RESULTS AND DISCUSSION

Water content and weight of a thousand seeds

The water contents of cultivars Carioca, Butter, Black and Red were of 16%, 14%, 15% and 13% respectively. According to Li et al. (2020) the moisture content is determinant for the physiological quality of the seeds, determining the viability and longevity of the seeds, since it directly interferes in the physiological processes, with the reduction of the quality of the seed, which can affect the vigor and even the germinative power. These authors also describe that the increase of the water content in the seeds of *Achnatherum inebrians* had a negative effect on the germination rate, in which seeds with moisture content of 21.5% presented only 16.5% of germination and seeds with 5, 5% humidity showed 86.5% germination. Such fact may be related to the highest number of abnormal seedlings in the control treatment of cultivar Carioca, which presented the highest water contents.

The weight of a thousand seeds (WTS) for cultivars Carioca, Butter, Black and Red were 196.47 g, 385.76 g, 181.15 g and 192.07 g respectively. And according to Carvalho and Nakagawa (2012) seeds with larger sizes, due to presenting higher amount of reserves, also present better physiologic quality such as vigor and germination, and such fact has been confirmed for various authors such as Barbosa et al. (2010) and Pádua et al. (2010). The characterization of the seeds through WTS was shown superior for the Butter cultivars.

Analysis of variance to identify the interaction between the factors aluminum doses and cultivars

The variance analysis (Table 1) enabled the identification of existence of significant interaction between the factors aluminum dosages (Al) and cultivars (Cv) for the features RL, LH, LE, RDM and AS. For the cultivar factor there has been significant difference for AS, RL, LE, LH, and RDM, and for Al factor there has been significant differences for the same features of the cultivar factor, except for RDM. The G and GSI characters have not presented significant difference for all treatments. A Scott-Knott average grouping test was performed, presented on Table 2 to differentiate the treatments arising from the A1 and Cultivar combination.

Stress by Al is specially designed to cause growth inhibition of the root, as a consequence of the alteration of different mechanisms and cellular structures. This one element affects the synthesis of DNA and the regulation of proteins that control the cell cycle. One Al toxicity causes disruption of reactive oxygen/ Ca^{2+} species homeostasis and Ca^{2+} mediated signaling, events considered key in inducing DNA damage or cell death in plants (Achary et al., 2013). In contrast,

Table 1: Analysis of variance and coefficient of variation (CV) for the physiological quality variables of four bean cultivars (Cv) treated with different concentrations of aluminum (Al)

FV	GL	Average square of variables							
		G	AS	GSI	RL	LH	LE	RDM	ADM
Cv	3	1.4	1.3908*	2.2746	0.35356***	116.932***	3.5381***	0.0312***	1.1346***
Al	4	0.5813	4.4431***	2.1601	2.91141***	55.636***	0.5593***	0.0103***	0.0056
Cv: Al	12	1.0979	0.4711*	3.8356	0.12491***	3.099*	0.0823***	0.0015***	0.004
Res	60	0.6583	0.2197	2.0905	0.03163	1.243	0.0337	0.0003	0.003
CV (%)		3.33	38.95	3.28	16.86	28.10	24.73	5.70	16.23

Significance of codes: **** 0.001 *** 0.01 ** 0.05 probability the F-test. Legend: germination (G); percentage of abnormal seedlings (AS); germination speed index (GSI); root length (RL); length of hypocotyls (LH); length of epicotyl (LE); root dry mass (RDM) and the dry mass of the shoot (ADM).

Table 2: Germination (G), percentage of abnormal seedlings (AS), germination speed index (GSI), root length (RL), length of hypocotyls (LH), length of epicotyl (LE), root dry mass (RDM) and the dry mass of the shoot (ADM) four bean cultivars (Cv) treated with different concentrations of aluminum (Al)

Treatments	Variables							
	G (%)	AS (%)	GSI	RL (cm)	LH (cm)	LE (cm)	RDM (mg)	ADM (mg)
B.00	98 ^{a1}	0 ^b	44.31 ^a	6.22 ^c	7.80 ^d	2.81 ^c	56.60 ^e	977.10 ^c
R.00	99 ^a	1 ^b	44.89 ^a	8.51 ^b	11.14 ^c	5.51 ^a	88.80 ^e	973.70 ^c
Bu. 00	98 ^a	1 ^b	44.56 ^a	6.47 ^c	5.47 ^e	1.09 ^d	108.90 ^d	2559.80 ^a
C.00	92 ^a	10 ^a	41.69 ^a	7.43 ^c	8.09 ^d	2.69 ^c	80.90 ^e	1033.60 ^c
B.2.5	99 ^a	0 ^b	43.68 ^a	8.81 ^b	8.95 ^d	3.15 ^c	107.20 ^d	999.00 ^c
R.2.5	96 ^a	13 ^a	43.52 ^a	10.72 ^a	13.62 ^b	6.24 ^a	122.80 ^d	936.70 ^c
Bu. 2.5	100 ^a	15 ^a	43.59 ^a	11.10 ^a	8.20 ^d	2.21 ^c	252.50 ^c	2176.50 ^b
C.2.5	99 ^a	14 ^a	44.18 ^a	10.29 ^a	12.49 ^c	2.88 ^c	133.90 ^d	986.90 ^c
B.5.0	97 ^a	11 ^a	44.10 ^a	5.79 ^c	12.47 ^c	3.87 ^b	138.90 ^d	897.10 ^c
R.5.0	96 ^a	17 ^a	43.44 ^a	7.49 ^c	16.07 ^a	6.11 ^a	137.50 ^d	904.70 ^c
Bu. 5.0	100 ^a	17 ^a	45.47 ^a	10.74 ^a	10.14 ^c	1.60 ^d	349.20 ^a	2229.30 ^b
C.5.0	100 ^a	15 ^a	45.72 ^a	6.57 ^c	13.88 ^b	2.76 ^c	128.40 ^d	961.20 ^c
B.7.5	99 ^a	21 ^a	44.89 ^a	4.60 ^d	11.36 ^c	3.22 ^c	123.20 ^d	838.40 ^c
R.7.5	95 ^a	25 ^a	42.93 ^a	5.19 ^d	13.22 ^b	4.03 ^b	124.8 ^d	960.40 ^c
Bu. 7.5	99 ^a	17 ^a	43.77 ^a	4.51 ^d	6.94 ^e	0.89 ^d	257.8 ^c	2180.60 ^b
C.7.5	97 ^a	17 ^a	44.31 ^a	4.96 ^d	11.92 ^c	2.68 ^c	145.00 ^d	1044.50 ^c
B.10	98 ^a	18 ^a	44.14 ^a	3.86 ^d	10.68 ^c	2.38 ^c	125.10 ^d	938.00 ^c
R.10	95 ^a	21 ^a	43.31 ^a	4.26 ^d	11.46 ^c	2.83 ^c	135.10 ^d	838.40 ^c
Bu. 10	98 ^a	18 ^a	44.56 ^a	5.24 ^d	5.83 ^e	0.47 ^d	291.90 ^b	2481.70 ^a
C.10	97 ^a	24 ^a	43.89 ^a	4.60 ^d	9.88 ^d	2.19 ^c	159.00 ^d	965.80 ^c

¹Means comparison within each column by the Scott-Knott's test ($p \leq 0,005$). Bean cultivars: B=black, R=red, Bu=butter and C=carrioca.

root transcriptome suggested efficient cell signaling and energy conservation key to aluminum toxicity tolerance in acidic soil adapted rice genotype (Jakoviak et al., 2020). The inhibition of root growth is the faster visible symptom Al toxicity in plants (Chen et al., 2011; Macedo et al., 2011a). Macedo and Lopes (2008) highlight that, if the aluminum penetrates only in the apoplast, not reaching the symplast, it may not compromise the cellular function.

Germination and seed vigor analysis

The percentage of germination and the index of germination speed not dissipation regardless of treatments, high percentages of germination, however it is observed that the use of the studied aluminum negatively affected the percentage of abnormal seedlings (Table 2). Rodrigues et al. (2017) studying the influence of 0, 300 and 600 μM of Al^{3+} on the emergence of seedlings of *Hancornia speciosa* found that the increase in settings

affected negatively by decreasing the emergence frequency, the emergence speed index and the root diameter of the seedlings, and the highest dose of Al^{3+} resulted in a 33.5% decrease in emergency when compared to the control group that presented approximately 90% emergency. Aluminum can cause toxicity in the embryonic axis of the seeds, compromising cell division and/or cell elongation, and subsequently, the appearance of abnormal seedlings (Ranal et al., 2016). However, the main effect is seen on the roots, and its length is the first parameter indicated to study in ecotoxicological tests (Senger et al., 2014).

For the root length variable, the highest values of the averages are found in the agreements with the concentration of 2.5 mg L^{-1} of aluminum sulfate, regardless of the cultivar, whereas the lowest values of the averages were found in the agreements with the standards of 7.5 and 10.0 mg L^{-1} of aluminum sulfate (Table 2). This

decrease in root growth with the increase in the aluminum sulfate dosage is also observed in Fig. 2. Silva and Matos (2016) state that it is possible that this metal, in low concentrations, plays a role as a micronutrient. However, as the accumulation of aluminum occurs preferentially in the root system of plants, their growth and development are reduced, increasing the diameter and decreasing the number of lateral roots (Macedo et al., 2011).

Toxicity to aluminum can cause several physiological and morphological effects on the plant, especially in young plants, having as an indicator of phytotoxicity the inhibition of root elongation, which can cause root atrophy, affecting the absorption of water and nutrients (Shetty et al., 2020). As an example, the increase in aluminum concentrations has exponentially reduced the length of the roots of *Jatropha curcas* genotypes (Martins et al., 2013), since aluminum compromises the vigor of the seeds, resulting in seedlings with the less developed root system, even in species tolerant (Rodrigues et al., 2019).

LH and LE variables, which represent the seedlings' aerial part, had their averages obtained by grouping into five and four distinct groups, respectively. All treatments involving the Red cultivars presented the higher averages for those two features, being significantly different from other treatments, except for treatments submitted to 10.0 mg L⁻¹ of aluminum, which, despite presenting the highest average, were not statistically different from the black cultivar for LH feature, and for LE feature there was no significant different for the black and carioca cultivars. Still regarding LH and LE features, it is observed that the cultivar butter presented the lowest averages regarding other treatments (Table 2). The symptoms described for the sprouts are usually minor and are considered an indirect response to the toxicity of Al, since they are reflections of the damage caused to the roots (Silva and Matos, 2016).

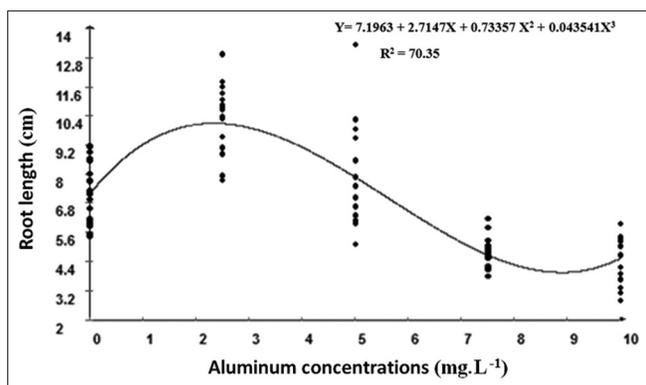


Fig 2. Cubic regression the length of the root (LR) four bean cultivars (Cv) treated with different concentrations of aluminum (Al).

For RDM variable, it is verified the formation of four averages groups (Table 2) and there has been an increase in the root's dry mass with the presence of aluminum, with the treatments for the cultivar butter presenting the highest averages, which were significantly different from the others. The dry mass increases as a result of the increase in the diameter of the roots, despite the smaller number and length of the roots, also a result of aluminum toxicity, which includes gross changes in the morphology of the root, with little formation and production of smaller, thick apices and dark in color (Wang et al., 2016). This is because, when aluminum is absorbed by the roots of plants in its toxic form, the inhibition of the growth of the primary and secondary root systems can occur through interference in the cell division of the root apices, associated with the ability of aluminum to bind to ionic transporters and the low nutrient content observed in species that are sensitive to this metal (Rodrigues et al., 2017).

The aerial part's dry mass (ADM) didn't present significant differences with A1 feature and interaction Al/Cv; the differences found were attributed to the factor cultivar (Table 1). The averages were separated into three groups (Table 2), and for all different aluminum concentrations, the averages were higher for the butter cultivar. Table 3 represents the correlation coefficients of the variable Al with the assessed variables, including the covariance between them. It is noted that correlations involving RL, LH, RDM and AS were significant, that is, have been changed due to the increase in the aluminum concentration and that the highest values were for correlations Al x RL and Al x AS, with the respective values of -0.6114 and 0.6157. There has been significant decrease in the root length and increase in the number of abnormal seedlings, due to the previously reported toxic effects of the aluminum.

Table 3: Covariance and Pearson's linear correlation coefficients between the physiological quality variables of seed four bean cultivars (Cv) treated with different concentrations of aluminum (Al)

Variables	Covariance	Correlation coef.
Al x RL	-2.178	-0.6114 **
Al x LH	0.5506	0.1314
Al x LE	-0.6142	-0.2633 *
Al x RDM	0.0398	0.3645 **
Al x ADM	-0.0362	-0.041
Al x G	-0.0253	-0.0205
Al x GSI	0.0929	0.0425
Al x AS	2.1899	0.6157 **

** *: Significant at 1 and 5% t test probability. Legend: germination (G); percentage of abnormal seedlings (AS); germination speed index (GSI); root length (RL); length of hypocotyls (LH); length of epicotyl (LE); root dry mass (RDM) and the dry mass of the shoot (ADM).

Table 4: Linear correlation coefficients of Pearson between the physiological quality variables of seeds of four bean cultivars (Cv) treated with different concentrations of aluminum (Al)

Variables	Aluminum concentration				
	0.0	2.5	5.0	7.5	10.0
RL x LH	0.8514	0.2307	-0.5716	0.8194	-0.8888
RL x LE	0.8382	0.1225	-0.4938	0.7409	-0.8593
RL x RDM	0.2107	0.6792	0.9456	-0.6097	0.9325
RL x ADM	-0.4391	0.5498	0.9476*	-0.5622	0.8595
RL x G	-0.0279	-0.1125	0.4618	-0.4945	0.2347
RL x GSI	-0.021	-0.1353	0.3644	-0.606	0.5216
RL x AS	0.2143	0.8268	0.3522	0.5915	-0.006
LH x LE	0.9935**	0.7502	0.875	0.9625*	0.9981**
LH x RDM	-0.2835	-0.5521	-0.8025	-0.9537*	-0.9823*
LH x ADM	-0.7714	-0.6858	-0.803	-0.9341	-0.9808*
LH x G	0.1442	-0.8018	-0.6311	-0.1023	-0.6035
LH x GSI	0.1154	0.2541	-0.5952	-0.122	-0.8364
LH x AS	-0.0028	0.6654	0.4728	0.6665	0.3934
LE x RDM	-0.2473	-0.5148	-0.6676	-0.9477*	-0.9734*
LE x ADM	-0.7182	-0.5619	-0.71	-0.9182	-0.9817*
LE x G	0.254	-0.9962**	-0.9219	-0.2169	-0.64
LE x GSI	0.2268	-0.4228	-0.9092	-0.161	-0.8628
LE x AS	-0.1113	0.2786	0.2027	0.8264	0.4413
RDM x ADM	0.7778	0.9831*	0.9962**	0.9964**	0.986*
RDM x G	0.1071	0.5562	0.5321	-0.1042	0.4471
RDM x GSI	0.1754	-0.223	0.4455	-0.1397	0.7243
RDM x AS	-0.0237	0.2306	0.043	-0.6258	-0.3609
ADM x G	0.2264	0.6128	0.5992	-0.1865	0.5228
ADM x GSI	0.2832	-0.3055	0.5155	-0.2175	0.7836
ADM x AS	-0.2658	0.0513	0.0832	-0.5645	-0.5086
G x GSI	0.9976**	0.3425	0.9943**	0.958*	0.9392
G x AS	-0.9814*	-0.3159	0.1383	-0.6364	-0.4924
GSI x AS	-0.9748*	0.2963	0.101	-0.4874	-0.5552

** *: Significant at 1 and 5% t test probability. Legend: germination (G); percentage of abnormal seedlings (AS); germination speed index (GSI); root length (RL); length of hypocotyls (LH); length of epicotyl (LE); root dry mass (RDM) and the dry mass of the shoot (ADM).

Correlation between the variables analyzed

The correlation study between the analyzed variables (Table 4) suggests that, in the absence of aluminum, the significant correlations were for LE x LH (0.9935), G x GSI (0.9976), G x AS (-0.9814) e GSI x AS (-0.9748). For dosage 2.5 mg L⁻¹ the significant correlations were only LE x G (-0.9962) and RDM x ADM (0.9831); For dosage 5.0 mg L⁻¹ the significant correlations were RL x ADM (0.9476), RDM x ADM (0.9962), G x GSI (0.9943); For dosage 7.5 mg L⁻¹ the significant correlations were LE x LH (0.9625), LH x RDM (-0.9537), LE x RDM (-0.9477), RDM x ADM (0.9964) and G x GSI (0.9580); For dosage 10.0 mg L⁻¹ the significant correlations were LH x LE (0.9981), LH x RDM (-0.9823), LH x ADM (-0.9808), LE x RDM (-0.9734), LE x ADM (-0.9817) and RDM x ADM (0.986). In its possible to observe, in Table 4, that all RDM x ADM correlations were significant by the t test in 5% probability level, except for the absence of aluminum, reflecting in the aluminum influence in the correlation root and aerial part dry mass.

Grouping analysis dendrogram

The dendrogram obtained in the grouping analysis (Fig 3) presents, in the vertical axis, the distance of Mahalanobis, in the horizontal axis the treatments, cultivar (B=black, R=red, Bu=butter and C=carioca) vs aluminum concentration (0.0, 2.5, 5.0, 7.5 and 10 mg L⁻¹), the cophenetic correlation coefficient was r = 0.88. In the dendrogram analysis a cutting line was traced, at approximately 20% homogeneity, with five distinct groups. It is worth noting that the different aluminum concentrations generated variability in treatments, since no group was formed with only one cultivar.

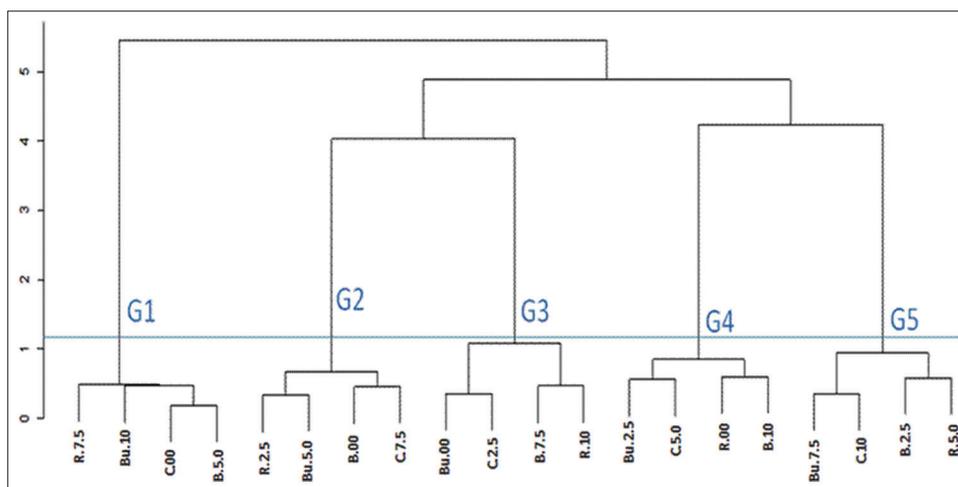


Fig 3. Dendrograma obtained by grouping method neighbor farther from the dissimilarity measures among 20 treatments, expressed by the Mahalanobis generalized distance.

The correlations, averages grouping test and grouping analysis results obtained in this study don't reflect the differences only due to different aluminum concentrations, thus it is important to highlight the importance of the wide genetic diversity of cultivars, especially when it comes to creole cultivars. Coelho et al. (2010) highlight that creole genotypes present a wide genetic base and are easily adapted to environmental conditions. None of the dosages used was lethal to the cultivars analyzed, thus suggesting they were rustic under the presence of different aluminum dosages; however, toxic effects were detected, expressed in reduced root length and increased number of abnormal seedlings.

CONCLUSION

The variables germination percentage and germination speed index were not affected by the toxic effect of aluminum. The variables percentage of abnormal seedlings, root length and shoot showed a significant reduction with the increase in aluminum sulfate concentrations, thus showing a greater correlation between them.

ACKNOWLEDGEMENTS

The authors thank the Federal University of Espírito Santo for providing facilities and equipment available for research; the Coordination for the Improvement of Higher Education Personnel (CAPES); the National Council for Scientific and Technological Development (CNPq) for financial support and research productivity grants to the third and last authors; to the Foundation for Research and Innovation Support of Espírito Santo (FAPES), for the granting research fee to the last author (FAPES Notice No. 19/2018 - Research Rate - FAPES Process N°. 82195510).

Authors' contributions

Josimar Aleixo da Silva: Methodology, preparation of the experiment; Allan Rocha de Freitas: Conceptualization, preparation of the experiment, writing-review; Rodrigo Sobreira Alexandre and José Carlos Lopes: Writing-review and editing, investigation, supervision; Paula Aparecida Muniz de Lima, Simone de Oliveira Lopes, Julcinara Oliveira Baptista and Tamyris Mello: Writing-review and editing.

REFERENCES

- Achary, V. M. M., N. L. Parinandi and B. B. Panda. 2013. Calcium channel blockers protect against aluminium-induced DNA damage and block adaptive response to genotoxic stress in plant cells. *Mutat. Res.* 751: 130-138.
- Barbosa, C. Z. R., O. J. Smiderle, J. M. A. Alves, A. A. Vilarinho and T. Sediyaama. 2010. Qualidade de sementes de soja BRS Tracajá, colhidas em Roraima em função do tamanho no armazenamento. *Rev. Ciênc. Agron.* 41: 73-80.
- Brasil. 2009. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Regras para análise de sementes. Brasília: Mapa/ACS, p. 399.
- Brasil. 2018. Ministério da Agricultura, Pecuária e Abastecimento. Plano Nacional Para o Desenvolvimento da Cadeia Produtiva do Feijão e Pulses. Available from: www.agricultura.gov.br/assuntos/camaras-setoriaisematicas/documentos/camaras-setoriais/feijao/2018/4a-re/minuta-pndcpfp-indicacao-contribuicoes-versao-02-02_2018.pdf [Last accessed on 2021 Mar 04].
- Campos-Vega, R., D. Oomah, G. Loarca-Piña and H. A. Vergara-Castañeda. 2013. Common beans and their non-digestible fraction: Cancer inhibitory activity: An overview. *Foods.* 2: 374-392.
- Carvalho, N. M and J. Nakagawa. 2012. Sementes: Ciência, Tecnologia e Produção. 5th ed. Jaboticabal: FUNEP.
- Chen, P., C. A. Sjogren, P. B. Larsen and A. Schnittger. 2019. A multi-level response to DNA damage induced by aluminium. *Plant J.* 98: 479-491.
- Cruz, C. D. 2016. Genes software-extended and integrated with the R, Matlab and Selegen. *Acta Sci. Agron.* 38: 547-552.
- Chen, Y. M., T. M. Tsao, C. C. Liu, K.C. Lin and M. K. Wang. 2011. Aluminium and nutrients induce changes in the profiles of phenolic substances in tea plants (*Camellia sinensis* CV TTES, No. 12 (TTE)). *J. Sci. Food Agric.* 91: 1111-1117.
- Coelho, C. M. M., M. R. Mota, C. A. Souza and D. J. Miquelluti. 2010. Potencial fisiológico em sementes de cultivares de feijão crioulo (*Phaseolus vulgaris* L.). *Rev. Bras. Sementes.* 32: 97-105.
- Coimbra, R. R., G. V. Miranda, C. D. Cruz, A. V. Melo and F. R. Eckert. 2010. Caracterização e divergência genética de populações de milho resgatadas do Sudeste de Minas Gerais. *Rev. Ciênc. Agron.* 41: 159-166.
- CONAB. 2020. Companhia Nacional de Abastecimento. Acompanhamento Safra Brasileira de Grãos. Vol. 7. Safra, Brazil.
- Domingues, A. M., E. Silva, G. Freitas, J. F. Ganança, H. Nóbrega, J. J. Slaski and M. A. P. Carvalho. 2013. Aluminium tolerance in bean traditional cultivars from Madeira. *Rev. Cienc. Agrar.* 36: 148-156.
- Eekhout, T., P. Larsen and L. Veylder. 2017. Modification of DNA checkpoints to confer aluminum tolerance. *Trends Plant Sci.* 22: 102-105.
- Freitas, L. B., D. M. Fernandes and S. C. M. Maia. 2012. Interação silício e alumínio em plantas de arroz de terras altas cultivada sem solo aluminico. *Rev. Bras. Ciênc. Solo.* 36: 507-515.
- Furlani, A. M. C. 2012. Nutrição mineral. In: G. B. Kerbauy, (Ed.), *Fisiologia Vegetal.* 2th ed. Rio de Janeiro: Guanabara Koogan.
- Jaskowiak, J., J. Kwasniewska, M. Szurman-Zubrzycka, M. Rojek-Jelonek, P. B. Larsen and I. Szarejko. 2020. Al-Tolerant Barley Mutant hvatr.g shows the ATR-regulated DNA damage response to maleic acid hydrazide. *Int J Mol Sci.* 21: 8500.
- Kumar, S and G. Pandey. 2020. Biofortification of pulses and legumes to enhance nutrition. *Heliyon.* 6: e036822.
- Kopittke, P. M., K. L. Moore, E. Lombi, A. Gianoncelli, B. J. Ferguson, F. P. C. Blamey, N. W. Menzies, T. M. Nicholson, B. A. Mckenna, P. Wang, P. M. Gresshoff, G. Kourousias, R. I. Webb, K. Green and A. Tollenaere. 2015. Identification of the primary lesion of toxic aluminum in plant roots. *Plant Physiol.* 167: 1402-1411.
- Li, X. Z., W. R. Simpson, M. L. Song, G. S. Bao, X. L. Niu, Z. H. Zhang, H. F. Xu, X. Liu, Y. L. Li and C. Li. 2020. Effects of seed moisture content and Epichloe endophyte on germination and physiology

- of *Achnatherum inebrians*. S. Afr. J. Bot. 134: 407-414.
- Macedo, C. M. P., J. C. Lopes, J. A. T. Amaral, A. F. A. Fonseca and J. F. T. Amaral. 2011. Tolerance of arabica coffee cultivars for aluminum in nutritive solution. Braz. Arch. Biol. Technol. 54: 885-891.
- Macedo, F. L., W. N. Pedra, S. A. Silva, M. C. V. Barreto and R. Silva-Mann. 2011. Efeito do alumínio em plantas de Pinhão-Manso (*Jatropha curcas* L.), cultivadas em solução nutritiva. Semina Ciênc Agrár. 32: 157-164.
- Machado, J. S., F. Steiner, F. Zoz, G. B. Honda and B. L. N. Oliveira. 2015. Effects of aluminum on seeds germination and Initial growth of physic nut seedlings. Rev. Agric. Neotrop. 2: 24-31.
- Maguire, J. D. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2: 176-177.
- Martins, L. D., J. C. Lopes, B. G. Laviola, T. V. Colodetti and W. N. Rodrigues. 2013. Selection of genotypes of *Jatropha curcas* L. for aluminium tolerance using the solution-paper method. Idesia. 31: 81-86.
- Nemli, S., T. K. Ascioğul, D. Ates, D. Esiyoirk and M. B. Taniyola. 2017. Diversity and genetic analysis through DARTseq in common bean (*Phaseolus vulgaris* L.) germplasm from Turkey. Turk. J. Agric. For. 41: 389-404.
- Osman, H. E., M. Al-Jabri, D. K. El-Ghareeb and Y. A. Al-Maroi. 2020. Impact of aluminum and zinc oxides on morphological characters, germination, metals accumulation and DNA in fenugreek (*Trigonella foenum-graecum*). J. Saudi Soc. Agric. Sci. 19: 510-520.
- Pádua, G. P., R. K. Zito, N. E. Arantes and J. B. França Neto. 2010. Influência do tamanho da semente na qualidade fisiológica e na produtividade da cultura da soja. Rev. Bras. Sementes. 32: 9-16.
- Palansooriya, K. N., S. M. Shaheen, S. S. Chen, D. C. W. Tsang, Y. Hashimoto, D. Hou, N. S. Bolan, J. Rinkleb and Y. S. Ok. 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review. Environ. Int. 134: 105046.
- Pitura, K. and S. D. Arntfield. 2019. Characteristics of flavonol glycosides in bean (*Phaseolus vulgaris* L.) seed coats. Food Chem. 272: 26-32.
- Rodrigues, A. A., S. C. Vasconcelos-Filho, C. L. Rodrigues, D. A. Rodrigues, G. P. Silva, J. F. Sales, K. J. T. Nascimento, E. M. G. Teles and L. S. Rehn. 2017. Aluminum influence on *Hancornia speciosa* seedling emergence, nutrient accumulation, growth and root anatomy. Flora. 236: 9-14.
- Rodrigues, A. A., S. C. Vasconcelos Filho, C. Müller, D. A. Rodrigues, J. F. Sales, J. Zuchi, A. C. Costa, C. L. Rodrigues, A. A. Silva and D. P. Barbosa. 2019. Tolerance of *Eugenia dysenterica* to aluminum: Germination and plant growth. Plants (Basel). 8: 317.
- Rosado, R. D. S., G. S. Fialho, B. A. S. Dias, T. B. Rosado, H. E. P. Martinez and B. G. Laviola. 2012. Screening *Jatropha* genotypes for aluminum tolerance using the solution-paper method. Semina Ciênc Agrár. 33: 1273-1280.
- Reis, A. R., L. A. M. Lisboa, H. P. G. Reis, J. P. Q. Barcelos, E. F. Santos, J. M. K. Santini, B. R. V. Meyer-Sand, F. F. Putti, F. S. Galindo, F. H. Kaneko, J. Z. Barbosa, A. P. Paixão, E. Furlani Junior, P. A. M. Figueiredo and J. Lavres. 2018. Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. Plant Physiol. Biochem. 130: 377-390.
- Ranal, M. A., C. M. Rodrigues, W. F. Teixeira, A. P. De Oliveira and R. Romero. 2016. Seed germination of *Microlicia fasciculata*, an apomictic and aluminium accumulator species: Unexpected intraspecific variability in a restricted Neotropical savanna area. Flora, 220: 8-16.
- R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.
- Scheffer-Basso, S. M. and B. C. Prior. 2015. Aluminum toxicity in roots of legume seedlings assessed by topological analysis. Acta Sci. Agron. 37: 61-68.
- Senger, E., A. Mohiley, J. Franzaring and J. M. Montes. 2014. Laboratory screening of aluminum tolerance in *Jatropha curcas* L. Ind. Crops Prod. 59: 248-251.
- Shetty, R., C. S. N. Vidya, N. B. Prakash, A. Lux and M. Vaculík. 2020. Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. Sci. Total Environ. 765: 142744.
- Silva, P. and M. Matos. 2016. Assessment of the impact of aluminum on germination, early growth and free proline content in *Lactuca sativa* L. Ecotoxicol. Environ. Saf. 131: 151-156.
- Silverio, J. M., C. C. Santos, R. S. Bernardes, G. M. Espíndola, H. L. Meurer and M. C. Vieira. 2021. Seed germination and vigor of *Arctium lappa* L. seedlings subjected to aluminium toxicity. Rev. Bras. Eng. Biosistemas. 15: 154-167.
- Wang, S., X. Ren, B. Huang, G. Wang, P. Zhou and Y. An. 2016. Aluminium-induced reduction of plant growth in alfalfa (*Medicago sativa*) is mediated by interrupting auxin transport and accumulation in roots. Sci. Reports. 6: 30079.
- Yamashita, O. M. and S. Guimaraes. 2011. Germination of *Conyza canadensis* and *Conyza bonariensis* Seeds as a function of light quality. Planta Daninha. 29: 737-743.