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GENOTYPES SELECTION FOR PLANT BIOASSAYS USING *LACTUCA SATIVA* L. AND *ALLIUM CEPA* L.

SIMONE CRISTINA DOS SANTOS[1], URSULÉIA APARECIDA DE OLIVEIRA[1], LUCIENE DE OLIVEIRA RIBEIRO TRINDADE[1], MATEUS DONIZETTI OLIVEIRA ASSIS[1], JOSÉ MARCELLO SALLABERT CAMPOS[2], EDUARDO GOMES SALGADO[3] AND SANDRO BARBOSA[1*]

[1]*Universidade Federal de Alfenas; Departamento de Ciências da Natureza, Alfenas-MG*
[2]*Universidade Federal de Juiz de Fora. Departamento de Biologia, Juiz de Fora-MG*
[3]*Universidade Federal de Alfenas. Departamento de Ciências Exatas, Alfenas-MG*
[*]*Corresponding author's email: sandro.barbosa@unifal-mg.edu.br (35) 3701-9696*

Abstract

Plant bioassays are considered an efficient, quick and inexpensive tool to monitor environmental interference caused by anthropogenic action, as well as to test the biological action of chemical substances. *Lactuca sativa* L. (Lettuce) and *Allium cepa* L. (Onion) are among the most used test organisms in plant bioassays; however, little is known about the behavior of different genotypes of these species within the bioassay dynamics. In this context, it is necessary to characterize and rank cultivars of these species that respond better to germination tests, initial growth and cytogenetic parameters for greater efficiency and reliability of the results from plant bioassays. 30 seeds were put in Petri dishes, moistened with 3.0 mL distilled water, placed in a germination chamber at 24ºC, with a 12-hour photoperiod. Germination percentage, germination speed index, number of normal seedlings, root length, shoot length, fresh and dry biomass, mitotic index, frequency of chromosomal abnormalities and DNA content were evaluated. Data were subjected to analysis of variance (ANOVA) at 5%, besides multicriteria analysis with the aid of TOPSIS. The most suitable cultivars for phytotoxicity and cytotoxotoxicity studies, in descending order, are Babá de Verão, Grand Rapids and Grandes Lagos (Lettuce) and Baia Periforme, Crioula and Vale Ouro (Onion). These genotypes were highly sensitive to characterize the toxicity of a chemical substance.

Key words: Cytogenetics, Lettuce. Onion. Phytotoxicity. Topsis.

Introduction

Biological assays can be used to identify and/or prove the interference of various chemical substances and thus characterize and quantify their positive or negative effects (Rizzo, 2011). Higher plants are excellent bioindicators of toxic activity, once they allow the evaluation of physiological, cytotoxic, genotoxic and mutagenic effects of environments contaminated by chemical substances. Among the species most suitable for plant bioassays are *Lactuca sativa* L. and *Allium cepa* L., due to peculiarities such as rapid and uniform germination, linear growth in a wide pH range, low sensitivity to osmotic potentials, ease of handling, material availability during the year, large number of dividing cells in the meristematic zone, small number of chromosomes, besides rapid responses to treatments and a high degree of sensitivity to toxicants (Cuchiara *et al.*, 2012; Simões *et al.*, 2013; Carvalho *et al.*, 2014).

Bioassays using plants as a test organism allow to evaluate phytotoxicity through the analysis of germination and initial growth (Ribeiro *et al.*, 2012; Carvalho *et al.*, 2014; Moraes *et al.*, 2015; Dar *et al.*, 2017), as well as the cytotoxotoxicity estimated by the increase or reduction in mitotic index and frequency of chromosomal abnormalities (Grippa *et al.*, 2010; Dias *et al.*, 2013; Neves *et al.*, 2016). These analyses may indicate aneugenic and/or clastogenic effects of chemical substances derived from the plant secondary metabolism (Martin-Puzon & Rivera, 2015; Bianchi *et al.*, 2015), besides contaminated water and industrial waste (Gomes *et al.*, 2015; Aguiar *et al.*, 2016).

The species in question have populations with well-defined characteristics denominated cultivars, which are grouped according to their phenotypic and genotypic descriptors. According to Xavier *et al.* (1995), cultivar is the designation corresponding to a genotype/phenotype, selected and properly registered, based on the characteristics that make it interesting for cultivation. Both the manifestation of production potential and the use of a cultivar as a target plant in bioassays depend on the interaction of its genome with the environment. Therefore, according to Lima (2004), the selection of the appropriate genetic material is a decisive tool for the success of the cultivation system and bioassays. Thus, it is of great relevance to identify and characterize, among Lettuce and Onion cultivars, those that have the best response regarding the endpoints used in plant bioassays.

No records of cultivar standardization for these species were found in the literature for allelopathy/toxicity studies; some studies do not report the cultivar used and, among those who mention them, there are differences in the use of different cultivars. Knowledge about the test organism (target species) and its behavior regarding the various genetic and physiological aspects is essential and, therefore, it is necessary to use methodologies that allow the characterization of genetically distinct materials within the target species.

Therefore, the objective of this study was to characterize, standardize, classify and validate, with a toxic substance, cultivars of Lettuce (*Lactuca sativa* L.) and Onion (*Allium cepa* L.), for germination, initial growth and cytogenetic parameters.

Table 1. *Lactuca sativa* L. cultivars obtained commercially.

Cultivar	Acronym	%G*	Shelf life	Purity (%)	Literature
Babá de Verão	BV	97	nov/16	100	4
Boston Branca	BB	83	oct/16	99.8	3
Delícia	Del	93	oct/16	100	1
Grand Rapids	GR	97	feb/16	100	48
Grandes Lagos	GL	98	dec/16	100	5
Maravilha de Inverno	MI	80	jul/16	99.8	1
Mimosa (Salad Bown)	Mi	94	nov/16	99.7	4
Mônica	Mo	98	jun/16	100	8
Quatro Estações	QE	90	dec/16	99.9	1
Rainha de Maio	RM	82	nov/16	99.8	6
Regina de Verão	RV	98	sep/16	99.8	1
Romana Branca de Paris	RBP	96	oct/16	99.9	1
Simpson Semente Preta	SSP	93	oct/16	99.9	3
Vitória de Verão	VV	93	sep/16	99.7	1

* Indication of germination according to supplier

Table 2. *Allium cepa* L. cultivars obtained commercially.

Cultivar	Acronym	%G*	Shelf life	Purity %	Literature
Baia Periforme	BP	80	feb/16	100	25
Baia Periforme Precoce	BPP	85	oct/16	100	0
Bola Precoce	BoP	80	feb/16	100	2
Conquista	Co	83	oct/16	100	0
Crioula	Cr	89	dec/16	99.9	0
Diamantina híbrida	Di	81	jun/16	99.9	0
Granex (híbrida)	Gr	80	jul/16	100	0
Ibiapaba (híbrida roxa)	Ib	80	jun/16	100	0
Primavera	Pr	85	jun/16	99.8	0
Roxa (Red Creole)	RRC	88	feb/16	100	0
Texas Grana 502	Te	85	jul/16	99.9	1
Vale Ouro IPA 11	VO	96	nov/16	100	0
White Creole	WC	82	apr/16	100	0

* Indication of germination according to supplier

Material and Methods

Fourteen *Lactuca sativa* L. cultivars and 13 *Allium cepa* L. cultivars, obtained commercially, were selected; they were investigated in the literature for their use in plant bioassays (Tables 1 and 2).

For each cultivar, 30 seeds were seeded in 70-mm Petri dishes, containing two disks of no. 2 Whitmann filter paper, moistened with 3 mL distilled water. The dishes were kept in a B.O.D. germination chamber (Solab Científica®), at 24°C, with a 12-hour photoperiod. The percentage of germination (%G) and germination speed index (GSI) were obtained by counting seeds with radicle protrusion. Thus, %G was evaluated at 24 and 48 hours for Lettuce and on the 3rd and 12th days for Onion, after the experiment was set. The GSI was analyzed every 4 hours up to 48 hours for Lettuce and, for Onion, every 8 hours up to 120 hours.

On the seventh day, the number of normal seedlings, that is, those with root and shoot, was determined, in addition to fresh biomass, using an analytical balance (Marte, AY 220). Root length (RL) and shoot length (SL) were measured from 10 visibly larger seedlings, with the aid of a digital caliper (Digimess, LR44). The seedlings were dried in a forced air oven (Nova Ética 400 ND) at 65°C for approximately 72 hours, until a constant mass was obtained; they were then weighed for the dry biomass.

For cytogenetic analyses, blades were prepared by the crushing method, adapted from Moraes *et al.* (2015) and Alvim *et al.* (2011). Root tips were collected at 24 hours (Lettuce) and 72 hours (Onion), respectively, fixed in Carnoy 3:1 and stored at -18°C. A total of 6000 cells were analyzed for each cultivar, and they were quantified at the different stages of the cell cycle to calculate mitotic index and chromosomal abnormalities (CA): micronucleus (MN), anaphase bridge (AB), telophase bridge (TB), C-metaphase (CM), stickiness (S) and lost chromosome (LC).

For quantification of the DNA content, totally expanded Lettuce young leaves and Onion roots were used. To estimate 2C DNA values, 20-30 mg of plant material, together with a reference standard (*Pisum sativum* cv. Ctirad for Lettuce and *Vicia faba* L. cv Inovec for Onion), were ground in the presence of LB01 buffer (1mL) to release the nuclei, according to the methodology cited by Dolezel *et al.* (1989). The crushed tissue was filtered and the removed nuclear suspension was stained with 25 µL of a solution consisting of propidium iodide and RNase. About 10,000 nuclei were analyzed for each sample using a FACSalibur cytometer (Becton Dickson). Each histogram generated in the cytometry was analyzed using the software WinMDI 2.8 Cell Quest. The values of 2C DNA for each sample were calculated by the relative fluorescence intensity of the sample relative to the internal reference standard.

Ranking data were submitted to analysis of variance (ANOVA) and the means were compared by the Scott-Knott test at 5% significance, using the software Sisvar (Ferreira, 2013).

A ranking of cultivars was also determined using the multicriteria analysis TOPSIS (Technique for Order Preference by Similarity), presented by Huang *et al.* (2011). The calculation involved in the analysis is described below:

Constructing a normalized matrix and the weighted normalized decision matrix (Equation 1), considering a normalized decision matrix, where $D = x_{mn}$:

$$a_{ij} = \frac{x_{ij}}{\sqrt{\sum_i^m \ x^2{}_{ij}}}, i = 1, \dots, m; j = 1, \dots, n.$$

(1)

The scores resulting from this calculation will be used to compare each action to an ideal (positive) and anti-ideal (negative) action, which is calculated by collecting the best and the worst performance in each criterion of the normalized decision matrix (Equation 2).

$$A^+ = \{b_1^+, b_2^+, \dots b_n^+\} = \{(\max_i b_{ij} | j \in K_b)\ (\min_i b_{ij} | j \in K_c)\}$$
$$A^- = \{b_1^-, b_2^-, \dots\ b_n^-\} = \{(\max_i b_{ij} | j \in K_b)\ (\min_i b_{ij} | j \in K_c)\} \quad (2)$$

where, K_b are the benefit criteria and K_c, the cost criteria.

Finally, the proximity to the ideal solution is calculated (Equation 3):

$$C_i = \frac{S_i^-}{S_i^- + S_i^+}, i = 1, \dots, m. \quad (3)$$

The positions of the alternatives are in accordance with the proximity to the ideal; therefore, the alternatives with greater C_i are supposedly more important and should be considered as priorities.

After ranking, the three best *L. sativa* and *A. cepa* cultivars were submitted to validation, to evaluate their behavior in the presence of a known toxic substance ($CdSO_4$). Thus, 30 seeds of each cultivar were put in the Petri dishes on a double layer of no. 2 Whitmann paper, containing 3 mL of $CdSO_4$ solution (500 µM) and distilled water as a negative control. The dishes were distributed in B.O.D (Solab Científica®) and maintained at 24°C with a 12-hour photoperiod. Evaluations for germination, initial growth and cytogenetic parameters were the same as those described in ranking and data were submitted to analysis of variance (ANOVA) at 5% significance.

Results

The results of germinative, initial growth and cytogenetic parameters, as well as the ranking obtained from the TOPSIS analysis of *Lactuca sativa* L. and *Allium cepa* L. cultivars, are given in Tables 3 and 4, respectively.

There was a significant difference ($p<0.05$) among *Lactuca sativa* L. cultivars in the germination (% age) at both 24 and 48 hours after culture (Table 3). It was observed that Babá de Verão, Grandes Lagos and Grand Rapids had a better performance both at 24 hours (about 93% to 98% germination), and 48 hours, totaling almost 100% germination. However, for % age, two cultivars did not present satisfactory germination: Maravilha de Inverno exhibited 14% and Mimosa, with only 2% germinated seeds at the first 48 hours. For *A. cepa* L. (Table 4), there was also a significant difference among cultivars, and Baia Periforme was statistically more significant than the others, with a result of 59% germination on the third day. On the twelfth day, Baia Periforme, along with Vale Ouro, Crioula and White Creole, were the most responsive to this parameter, with averages of approximately 90% germination. Cultivars Roxa Red Criole and Granex did not show germination on the third day. However, on the twelfth day, about 24% and 0.8% germination was observed, respectively.

For Germination Speed Index (GSI) of lettuce cultivars, Grand Rapids showed a higher average (17.6), while Mimosa and Maravilha de Inverno presented results statistically equal, with very low averages (0.09 and 0.66, respectively): they were, therefore, the latest. On the other hand, for Onion, the highest GSI values were obtained for Baia Periforme, Crioula, Diamantina, Vale Ouro and White Creole, which did not differ statistically, with indices of approximately 4 to 5. Cultivar Granex had the lowest GSI, with an average of 0.03.

In Lettuce, Grand Rapids, Grandes Lagos and Babá de Verão, as in the previous parameters, there was a greater percentage of normal seedlings (NS), about 95%. The NS for Mimosa and Maravilha de Inverno was the lowest observed (1.6%), which was already predicted, due to low germinability. In Onion, Baia Periforme, Vale Ouro, Crioula and White Creole presented the highest NS values. Cultivar Granex presented NS equal to zero and was, therefore, the less responsive to this parameter.

It was observed that, among *L. sativa* cultivars, Grand Rapids had the highest root length (37.3 mm) and shoot length (30.6 mm), and that cultivar Maravilha de Inverno did not show any growth. In *A. cepa*, Baia Periforme, Bola Precoce and Vale Ouro were statistically equal to each other, exhibiting the highest root length averages (8.8 to 9.9 mm). Baia Periforme, Bola Precoce and Crioula were statistically equal, presenting the highest shoot lengths (35 to 38 mm). Granex did not show root and shoot growth.

Lactuca sativa cv Babá de Verão stood out, showing the highest value (0.588g) for fresh biomass (FB). Mimosa and Maravilha de Inverno, as well as in the other variables, had very low FB values (0.091 and 0.094g, respectively). In relation to dry biomass (DB), Babá de Verão and Grand Rapids were statistically equal, with the highest averages (0.028g and 0.030g, respectively), and Maravilha de Inverno exhibited the lowest average (0.01g). For *Allium cepa* L., Baia Periforme and White Creole did not differ statistically, presenting the highest FB averages. Granex and Roxa Red Creole were also less responsive to this parameter, with the lowest values (0.19g). Ibiapaba was the cultivar with the highest DB accumulation (0.101g) and Primavera had the lowest value (0.058g).

Table 3. Ranking of Lettuce cultivars obtained from germination, initial growth, cytogenetic parameters and TOPSIS analysis.

Cult.	G24h (%)	G48h (%)	GSI	NS (%)	RL (mm)	SL (mm)	FB (g)	DB (g)	MI (%)	DNA (pg)	TOPSIS	R.
BV	98^{a}	100^{a}	13.68^{c}	95^{a}	28.79^{b}	24.15^{c}	0.588^{a}	0.028^{a}	35.8^{a}	6.18^{a}	0.99589	1º
GR	98^{a}	97^{a}	17.6^{a}	94^{a}	37.3^{a}	30.61^{a}	0.398^{d}	0.030^{a}	36^{a}	6.10^{a}	0.99561	2º
GL	93^{a}	99^{a}	16.13^{b}	95.8^{a}	25.83^{c}	22.40^{c}	0.544^{b}	0.020^{b}	34.9^{a}	6.23^{a}	0.99382	3º
BB	79^{b}	88^{b}	9.79^{d}	82.5^{b}	28.51^{b}	24.10^{c}	0.452^{c}	0.020^{b}	34.5^{a}	6.20^{a}	0.98955	4º
VV	74^{b}	90^{b}	9.92^{d}	80^{b}	21.47^{d}	30.46^{a}	0.420^{d}	0.020^{b}	29.5^{b}	6.23^{a}	0.98816	5º
RM	15^{g}	69^{c}	3.71^{f}	53^{c}	17.49^{e}	19.79^{d}	0.237^{g}	0.018^{b}	21.6^{c}	6.26^{a}	0.98071	6º
Mo	54^{d}	85.8^{b}	6.98^{e}	83^{b}	24.50^{c}	21.30^{d}	0.320^{e}	0.020^{b}	15^{d}	6.11^{a}	0.97833	7º
RV	22.5^{f}	63^{c}	3.77^{f}	33^{d}	12.91^{f}	19.75^{d}	0.198^{h}	0.018^{b}	27^{b}	6.33^{a}	0.97761	8º
SSP	30^{f}	62^{c}	4.19^{f}	40^{d}	16.28^{e}	20.84^{d}	0.275^{f}	0.022^{b}	26^{b}	6.21^{a}	0.95797	9º
Del	22.5^{f}	55^{c}	4.03^{f}	37.5^{d}	16.74^{e}	17.95^{e}	0.200^{h}	0.018^{b}	29.7^{b}	6.20^{a}	0.95252	10º
RBP	44^{e}	85^{b}	6.29^{e}	79^{b}	20.67^{d}	23.00^{c}	0.443^{c}	0.023^{b}	28.5^{b}	6.33^{a}	0.94701	11º
QE	62.5^{c}	90.8^{b}	7.19^{e}	87.5^{b}	19.90^{d}	26.93^{b}	0.448^{c}	0.020^{b}	19^{c}	6.24^{a}	0.94403	12º
MI	1.6^{h}	14^{d}	0.66^{g}	1.6^{e}	0.00^{h}	0.00^{c}	0.090^{i}	0.010^{c}	8^{e}	6.24^{a}	0.78038	13º
Mi	0^{h}	2.5^{e}	0.09^{g}	1.6^{e}	4.82^{g}	0.00^{c}	0.095^{i}	0.023^{b}	14.7^{d}	6.10^{a}	0.75256	14º
CV	11.18	9.69	12.17	10.9	8.38	9.34	7.09	19.63	12.66	-	-	-

*Columns followed by distinct letters differ by the Scott-Knott test at 5% significance. Cult. - Cultivars, G - Percentage of Germination, GSI - Germination Speed Index, NS - Number of Normal Seedlings, RL - Root Length, SL - Shoot length, FB - Fresh Biomass, DB - Dry Biomass, MI - Mitotic Index, DNA - DNA Content, TOPSIS - Technique for Order Preference by Similarity, R. - Ranking, CV - Coefficient of Variation

Table 4. Ranking of Onion cultivars obtained from germination, initial growth, cytogenetic parameters and TOPSIS analysis.

Cult.	G3°d (%)	G12°d (%)	GSI	NS (%)	RL (mm)	SL (mm)	FB (g)	DB (g)	MI (%)	DNA (pg)	TOPSIS	R.
BP	59^{a}	93^{a}	5.18^{a}	86.7^{a}	8.85^{a}	37.47^{a}	0.507^{a}	0.083^{c}	14.8^{a}	34.78^{a}	0.91064	1º
VO	41^{b}	91.7^{a}	4.72^{a}	85.8^{a}	9.92^{a}	31.93^{b}	0.438^{b}	0.074^{d}	10^{b}	34.80^{a}	0.80149	2º
WC	31^{c}	89 ^{a}	4.33^{a}	81.7^{a}	5.11^{c}	29.53^{b}	0.493^{a}	0.086^{b}	9.9^{b}	-	0.72115	3º
Cr	45^{b}	90^{a}	4.68^{a}	77^{a}	4.35^{d}	35.44^{a}	0.389^{c}	0.070^{e}	8.6^{c}	34.57^{a}	0.68723	4º
Pr	42^{b}	81.7^{b}	4.70^{a}	70^{b}	4.99^{c}	30.74^{b}	0.355^{c}	0.058^{f}	6^{d}	34.80^{a}	0.62817	5º
Di	49.7^{b}	85.8^{b}	4.58^{a}	70.8^{b}	7.36^{b}	28.52^{c}	0.371^{c}	0.087^{b}	4^{e}	34.76^{a}	0.51374	6º
BPP	32^{c}	72.3^{c}	3.65^{b}	58^{c}	4.12^{d}	26.29^{c}	0.332^{d}	0.066^{e}	2^{f}	34.83^{a}	0.47722	7º
Co	13^{e}	67.5^{c}	2.88^{c}	62^{c}	5.77^{c}	20.31^{d}	0.307^{d}	0.080^{c}	2.8^{f}	34.60^{a}	0.43495	8º
BoP	4^{e}	45^{e}	0.95^{d}	31.7^{d}	9.29^{a}	38.64^{a}	0.306^{d}	0.069^{e}	3^{f}	34.70^{a}	0.40835	9º
TG	23^{d}	55^{d}	2.88^{c}	30^{d}	5.20^{c}	17.00^{e}	0.304^{d}	0.074^{d}	2^{f}	34.67^{a}	0.39306	10º
Ib	13^{e}	31^{f}	1.31^{d}	12^{f}	6.84^{b}	16.58^{e}	0.256^{e}	0.101^{a}	0.3^{g}	34.54^{a}	0.27213	11º
RRC	0^{e}	24^{g}	0.93^{d}	19^{e}	3.55^{d}	11.17^{f}	0.192^{f}	0.080^{c}	0^{g}	34.78^{a}	0.18025	12º
Gr	0^{e}	0.8^{h}	0.03^{e}	0^{g}	0.00^{e}	0.00^{g}	0.197^{f}	0.090^{b}	0^{g}	34.67^{a}	0.07399	13º
CV	12.5	6.42	11.22	12.17	12.34	9.63	9.91	3.6	13.05	-	-	-

*Columns followed by distinct letters differ by the Scott-Knott test at 5% significance. Cult. - Cultivars, G - Percentage of Germination, GSI - Germination Speed Index, NS - Number of Normal Seedlings, RL - Root Length, SL - Shoot length, FB - Fresh Biomass, DB - Dry Biomass, MI - Mitotic Index, DNA - DNA Content, TOPSIS - Technique for Order Preference by Similarity, R. - Ranking, CV - Coefficient of Variation.

Table 5. Chromosomal abnormalities in root cells from *Lactuca sativa* L. seeds exposed to distilled water.

Cultivars	MN	AB	TB	CM	S	LC	FCA (%)
BB	0	1	6	13	14	10	0.73
BV	6	1	8	13	16	21	1.08
Del	8	1	4	14	8	4	0.65
GL	3	0	0	8	8	7	0.43
GR	4	1	15	7	7	17	0.85
MI	3	0	2	1	2	3	0.18
Mi	0	0	0	0	1	1	0.03
Mo	12	1	12	2	7	9	0.71
QE	13	2	12	6	1	6	0.66
RBP	2	1	1	12	16	9	0.68
RM	4	1	1	5	1	8	0.33
RV	0	1	0	6	4	4	0.25
SSP	10	0	9	1	17	17	0.90
VV	0	2	2	5	21	9	0.65

MN - Micronucleus; AB - Anaphase bridge; TB - Telophase bridge; CM - C-metaphase; S - Stickiness; LC - Lost chromosomes; FCA (%) - Frequency of chromosomal abnormalities. Cytogenetic analyses were performed counting 6000 cells per treatment

Table 6. Chromosomal abnormalities in root cells from *Allium cepa* L. seeds exposed to distilled water.

Cultivars	MN	AB	TB	CM	S	LC	FCA (%)
BP	10	0	1	2	20	1	0.57
BPP	8	0	0	1	11	2	0.37
BoP	0	0	0	0	4	0	0.07
Co	4	0	0	1	5	0	0.17
Cr	9	0	0	5	13	0	0.45
Di	2	1	0	1	0	2	0.10
Gr	0	0	0	0	0	0	0
Ib	0	0	0	0	2	0	0.03
Pr	5	2	0	6	14	1	0.47
RRC	0	0	0	0	0	0	0
TG	12	0	0	0	3	2	0.28
VO	6	1	1	2	14	4	0.47
WC	2	0	2	3	6	0	0.38

MN - Micronucleus; AB - Anaphase bridge; TB - Telophase bridge; CM - C-metaphase; S - Stickiness; LC - Lost chromosomes; FCA (%) - Frequency of chromosomal abnormalities. Cytogenetic analyses were performed counting 6000 cells per treatment

Regarding mitotic index (MI), lettuce cultivars Boston Branca, Babá de Verão, Grandes Lagos and Grand Rapids showed the highest cell division rate (34 to 36%). In Onion, the cultivar with the highest MI was Baia Periforme, with 14.8% cells in division.

The results obtained by flow cytometry for the quantification of DNA content (pg) showed that there was no statistical difference between the values for *Lactuca sativa* L. and *Allium cepa* L. cultivars, ranging from 6.10 to 6.33 and 34.5 to 34.8; respectively.

In the multicriteria analysis (Technique for Order Preference by Similarity - TOPSIS), a ranking of Lettuce and Onion cultivars was obtained, in which Babá de Verão and Baia Periforme presented results closest to the ideal positive solution (0.99589 and 0.91064, respectively). Cultivars Mimosa (Lettuce) and Granex (Onion) showed the worst performance in the evaluated criteria, approaching the ideal negative solution, with 0.75256 and 0.07399, respectively.

In the analysis of chromosomal abnormalities (Tables 5 and 6), cultivar Babá de Verão (Lettuce) presented the highest frequency of abnormalities (FCA), 1.08%, with a predominance of lost chromosome, while Baia Periforme (Onion) presented the highest FCA (0.57%), with the highest percentage of stickiness. The abnormalities of each species are shown in Figure 1.

From the ranking obtained in the analysis of variance and TOPSIS, the three cultivars most responsive to the tested variables were: Babá de Verão, Grandes Lagos and Grand Rapids for *Lactuca sativa* L., and Baia Periforme, Vale Ouro and White Creole for *Allium cepa* L. They were validated by exposure to a known toxic substance, $CdSO_4$, tested in previous studies at a concentration of 500µM (Tables 7 and 8). Cultivar White Creole was not found commercially during experiment setting, making it impossible to include it in the analyses. Therefore, Crioula (4th best in the ranking) was chosen to proceed in the validation experiments.

For *Lactuca sativa* L., there was no significant difference between % age of cultivars exposed to the control and $CdSO_4$, both at 24 and 48 hours. There was a delay in the germination speed of Babá de Verão and Grandes Lagos submitted to the treatment in relation to the control, which was not observed for Grand Rapids. For the other parameters, except DB, there was a significant reduction in cultivar average, when exposed to $CdSO_4$ in relation to the control. Therefore, the treatment with Cadmium influenced the growth of Lettuce seedlings (Fig. 2A).

For the three *Allium cepa* cultivars, the seeds exposed to Cadmium presented a reduction in % age in relation to the control, both at 72 and 96 hours. Baia Periforme and Crioula exposed to the metal had reduced GSI, compared to the control, whereas for Vale Ouro, the metal accelerated seed germination.

Only cultivar Crioula presented sensitivity to Cadmium for RL, since the metal inhibited root growth, when compared to the control. On the other hand, the exposure to the metal did not influence the SL of Crioula, whereas for the other cultivars, there was a difference between the treatment with the metal and the control (Fig. 2B).

For FB and DB, there was no statistical difference between the treatment with Cadmium and the control for *A. cepa*.

For Baia Periforme and Vale Ouro, Cadmium inhibited cell division in relation to the control, a fact not observed in cultivar Crioula, in which the metal stimulated cell division, resulting in a MI higher than the control.

Regarding chromosomal abnormalities (Tables 9 and 10), the treatment with Cadmium did not alter the number of CA in Babá de Verão and Grandes Lagos in relation to the control. However, for Grand Rapids, the control had a higher percentage of abnormalities (0.47%), since the number of dividing cells in roots exposed to Cadmium was very low. It is also emphasized that C-metaphases and Stickiness were the most frequent abnormalities.

In *A. cepa* cv Baia Periforme, the frequency of chromosomal abnormalities was not influenced by Cadmium. In Crioula, the exposure to $CdSO_4$ led to an increase in FCA while, in Vale Ouro, the treatment reduced this frequency.

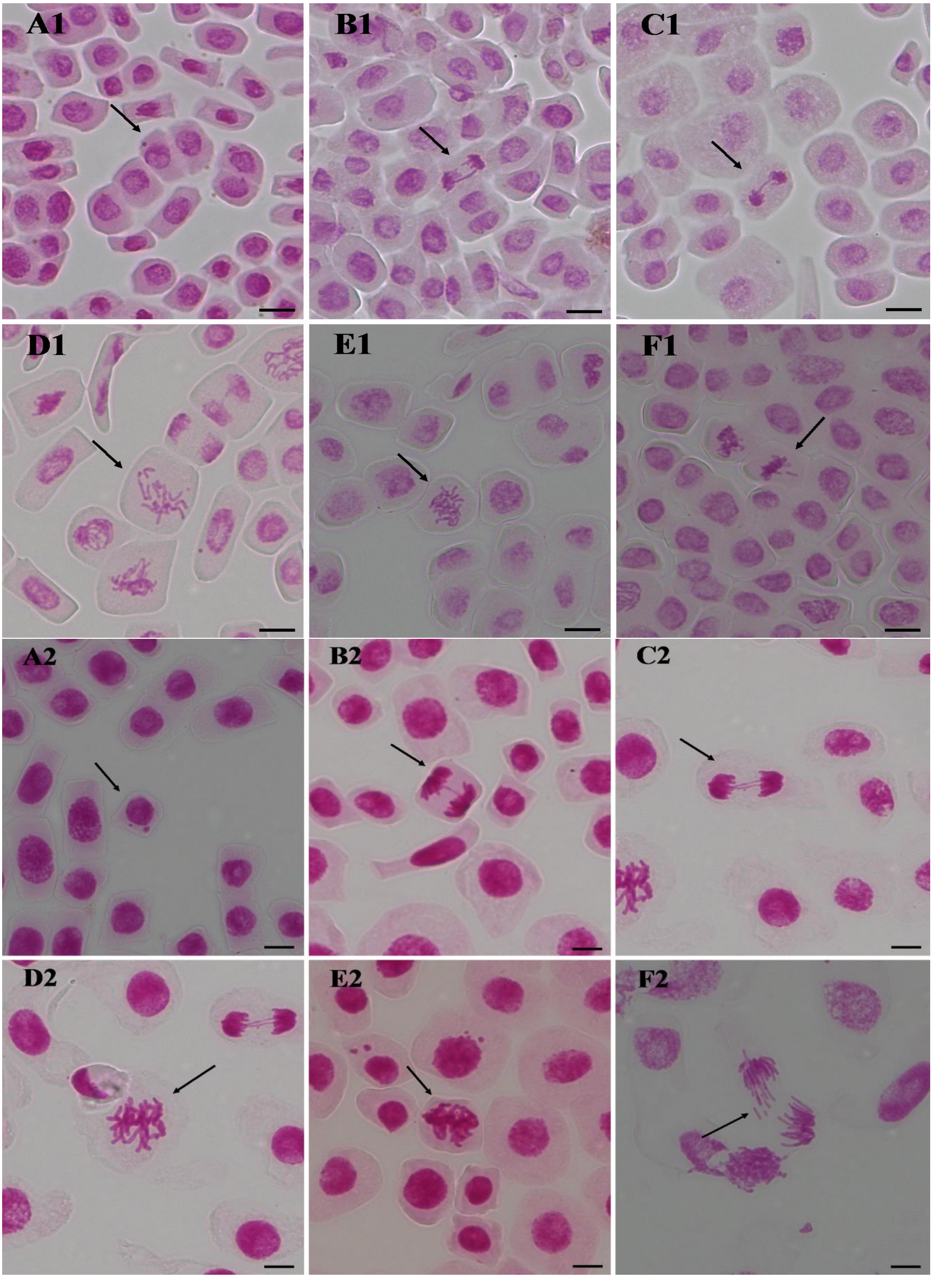

Fig. 1. Chromosomal abnormalities observed in meristematic cells of *L. sativa* (1) and *A. cepa* (2) exposed to distilled water. (A1 and A2) Micronucleus; (B1 and B2) Anaphase Bridge; (C1 and C2) Telophase Bridge; (D1 and D2) C-Methaphase; (E1 and E2) Stickiness; (F1 and F2) Lost Chromosome.

Fig. 2. Action of a toxic substance in the plant bioassay. A) Lettuce exposed to distilled water (control) and $CdSO_4$ solution. B) Onion exposed to distilled water (control) and $CdSO_4$ solution.

Table 7. Germination, initial growth and cytogenetic variables for different *Lactuca sativa* L. cultivars exposed to distilled water as a negative control and to $CdSO_4$ solution (500 µM).

Cultivar		G2 4 h (%)	G 48 h (%)	GSI	NS (%)	RL (mm)	SL (mm)	FB (g)	DB (g)	MI (%)
BV	Control	94[a]	98.9[a]	21.24[a]	93[a]	23.13[a]	21.15[a]	0.430[a]	0.023[a]	23[a]
	$CdSO_4$	94[a]	97.8[a]	16.89[b]	12[b]	2.62[b]	9.28[b]	0.230[b]	0.020[a]	3.4[b]
GL	Control	95[a]	100[a]	22.56[a]	95[a]	26.03[a]	20.69[a]	0.473[a]	0.026[a]	28[a]
	$CdSO_4$	96[a]	100[a]	17.38[b]	10[b]	3.00[b]	7.94[b]	0.240[b]	0.020[a]	1.8[b]
GR	Control	93[a]	97.8[a]	16.46[a]	94[a]	37.85[a]	15.45[a]	0.413[a]	0.030[a]	31[a]
	$CdSO_4$	92[a]	97.8[a]	16.28[a]	3.3[b]	3.43[b]	7.59[b]	0.203[b]	0.030[a]	0.03[b]
CV		6.10	4.04	7.53	8.04	15.46	12.43	4.44	10.6	13.54

*Columns followed by distinct letters differ by the Scott-Knott test at 5% significance. G - Percentage of Germination, GSI - Germination Speed Index, NS - Number of Normal Seedlings, RL - Root Length, SL - Shoot length, FB - Fresh Biomass, DB - Dry Biomass, MI - Mitotic Index, CV - Coefficient of Variation

Table 8. Germination, initial growth and cytogenetic variables for different *Allium cepa* L. cultivars exposed to distilled water as a negative control and to $CdSO_4$ solution (500 µM).

Cultivar		G 72 h (%)	G 96 h (%)	GSI	NS (%)	RL (mm)	SL (mm)	FB (g)	DB (g)	MI (%)
BP	Control	53[a]	72[a]	2.16[a]	81[a]	11.18[a]	35.68[a]	0.380[a]	0.090[a]	22[a]
	$CdSO_4$	31[b]	38[b]	1.22[b]	74[b]	10.11[a]	26.43[b]	0.335[a]	0.090[a]	17[b]
Cr	Control	38[a]	52[a]	1.80[a]	22[a]	7.89[a]	24.84[a]	0.267[a]	0.060[a]	8.9[b]
	$CdSO_4$	31[b]	35[b]	1.38[b]	15[b]	6.15[b]	24.35[a]	0.235[a]	0.060[a]	13[a]
VO	Control	23[a]	35[a]	1.10[b]	18[a]	5.99[a]	17.69[a]	0.200[a]	0.063[a]	14[a]
	$CdSO_4$	16[b]	19[b]	1.31[a]	11[b]	5.03[a]	11.01[b]	0.229[a]	0.080[a]	2.04[b]
CV		8.88	10.46	6.50	11.16	7.77	7.89	11.73	3.19	5.43

*Columns followed by distinct letters differ by the Scott-Knott test at 5% significance. G - Percentage of Germination, GSI - Germination Speed Index, NS - Number of Normal Seedlings, RL - Root Length, SL - Shoot length, FB - Fresh Biomass, DB - Dry Biomass, MI - Mitotic Index, CV - Coefficient of Variation

Table 9. Chromosomal abnormalities in root cells from *Lactuca sativa* L. seeds exposed to 500 µM cadmium solution and distilled water as a negative control.

Cultivar		MN	AB	TB	CM	S	LC	FCA (%)
BV	Control	0	0	0	3	9	1	0.22
	$CdSO_4$	0	0	2	12	1	1	0.27
GL	Control	1	0	0	1	7	5	0.23
	$CdSO_4$	0	0	0	9	7	0	0.27
GR	Control	0	1	0	0	26	1	0.47
	$CdSO_4$	1	0	0	1	0	0	0.03

MN - Micronucleus; AB - Anaphase bridge; TB - Telophase bridge; CM - C-metaphase; S - Stickiness; LC - Lost chromosomes; FCA (%) - Frequency of chromosomal abnormalities. Cytogenetic analyses were performed counting 6000 cells per treatment

Table 10. Chromosomal abnormalities in root cells from *Allium cepa* L. seeds exposed to 500 µM cadmium solution and distilled water as a negative control.

Cultivar		MN	AB	TB	CM	S	LC	FCA (%)
BP	Control	10	1	0	0	17	2	0.50
	$CdSO_4$	4	0	1	0	21	4	0.50
Cr	Control	12	2	2	0	16	1	0.52
	$CdSO_4$	32	0	0	1	31	2	1.12
VO	Control	44	2	4	2	15	23	1.50
	$CdSO_4$	1	0	0	0	3	0	0.07

MN - Micronucleus; AB - Anaphase bridge; TB - Telophase bridge; CM - C-metaphase; S - Stickiness; LC - Lost chromosomes; FCA (%) - Frequency of chromosomal abnormalities. Cytogenetic analyses were performed counting 6000 cells per treatment

Discussion

Lettuce cultivar Grand Rapids among all the genotypes was most used in the literature as a test organism in plant assays (Catão *et al*., 2014; Paula *et al*., 2015, Santos *et al*., 2015), compared to other cultivars. From a total of 178 articles analyzed (from 2004 to 2016), 48 report the use of Grand Rapids in phytotoxicity experiments. In this study, this cultivar presented excellent performance, especially for germination percentage (% G), which was considered early, according to articles that evaluate % G at 24 and 48 hours (Moraes *et al*., 2015; Mecina *et al*., 2016). Although Grand Rapids is the most reported, Babá de Verão obtained the highest averages for most parameters in the analysis of variance and stood out first in the TOPSIS ranking.

Allium cepa L. is one of the most used species in plant bioassays. However, few articles identify the genotype used as a target plant. From 120 articles selected (from 2004 to 2016), 25 report Baia Periforme in their research. This cultivar stands out, once it allows a homogeneous analysis, both from a genetic and a physiological point of view, which provides greater reliability for the performed bioassays (Santos *et al*., 2011; Grisi *et al*., 2013; Lacerda *et al*., 2014; Peres *et al*., 2015; Rodrigues *et al*., 2015; Martins *et al*., 2016). In this paper, this cultivar stood out in all parameters analyzed, and was superior to the others for %G on the 3rd day, as well as for mitotic index (MI). Ragassi *et al*. (2012) point out that some Brazilian Onion cultivars have contributions from Baia Periforme in their genetic basis. Cultivars such as Vale Ouro IPA 11, Crioula, Bola Precoce, included in this study, are the result of breeding programs that used Baia Periforme in the cross breeds (Leite *et al*., 2009), which corroborates some of these genotypes among the first in rankings.

A high % age, as well as high values of germination speed index (GSI), are important parameters to be considered, especially in the negative control, in studies evaluating the influence of allelochemicals and/or potentially toxic substances (Kikuti & Marcos Filho, 2012; Carvalho *et al*., 2014; Catão *et al*., 2014; Peres *et al*., 2015). The high germinative capacity (rate equal to or higher than 80%) of the seeds in the control group of an experiment allows greater precision, reliability and data validation. Nascimento (2002) states that such parameters provide the researcher with a better knowledge of the physiological performance of species in phytotoxicity bioassays, allowing more consistent and rapid responses. Vieira & Carvalho (1994) point out that experimental results from the use of genotypes with low percentage of germination exposed to a toxic agent can be underestimated. Therefore, toxicity experiments using a suitable cultivar allow to infer that the low % G and GSI values found in the treatments are most frequently associated with the toxic effect of the tested substance. Thus, even with the essential use of the negative control, the use of cultivars with a high germination percentage is essential.

The cultivars with the worst performance for % age and GSI in *L. sativa* were Mimosa and Maravilha de Inverno and, in *Allium cepa* L., Granex and Roxa Red Creole; thus, they are not suitable for plant bioassays. They did not correspond to the %age indicated by the supplier and, according to Nascimento (2002), this may be related to seed vigor, which is defined as properties that determine the potential for a rapid and uniform emergence, providing a normal seedling development under different cultivation conditions. Therefore, vigor is also related to GSI, since it calculates the average number of seeds germinated in a time interval. It is noteworthy that optimal germination conditions were given to these Lettuce and Onion genotypes and, probably due to the low vigor of their seeds, their performance in the experiments was compromised.

The cultivars that stood out for root length (RL) and shoot length (SL) were Grand Rapids (Lettuce) and Baia Periforme, Bola Precoce and Vale Ouro (Onion). In a plant bioassay, RL is one of the most cited endpoints (Lutterbeck *et al*., 2015; Freitas *et al*., 2016; Verdejo *et al*., 2016), due to its simplicity, speed and sensitivity. Park *et al.* (2016) studied lettuce cultivated in hydroponics, and reported the importance of RL as an appropriate endpoint in the evaluation of toxic substances at different concentrations in bioassays for the effect of metals, wastewater and other complex substances. According to Taiz & Zeiger (2013), it is known that the root function is to provide support and absorption of nutrients by the plant, which are used for its development. Thus, it can be inferred that the larger the root length, the larger its absorption surface. Ratsch (1986) states that the physiological effects of metals on plants are closely linked to the absorption and accumulation of toxic substances, and the first organ affected is the root, once it is more in contact with the tested substance. Thus, a significant reduction in root length is considered to be a valid and sensitive response to the effects of toxicants, when compared to the initial germination process (imbibition) and shoot growth. However, SL analysis is also important, since toxic agents may not affect root germination and growth, but may alter leaf growth and development patterns and, consequently, alter photosynthetic (carbon allocation) and/or respiratory rates in the test plant. In a study on the growth of volatile organic compounds emitted by bacteria in *L. sativa* conducted by Fincheira *et al.* (2016), SL of lettuce seedlings was more affected than RL, when submitted to one of the treatments.

Normal seedlings were those with functional root and shoot, and cultivars Grandes Lagos, Babá de Verão and Grand Rapids (Lettuce) and Baia Periforme, Crioula, Vale Ouro and White Creole (Onion) were the most outstanding in this parameter. Mimosa and Maravilha de Inverno (*L. sativa* L.) and Granex (*A. cepa* L.) presented low frequencies of normal seedlings, which was already expected, due to the low %G. This is corroborated by Nascimento (2002), since seeds with low vigor can provide an increase in the number of abnormal seedlings.

Leme & Marin-Morales (2009) report that meristematic plant root cells are excellent indicators of cytogenotoxic effects and several researchers have used mitotic index (MI) as one of the main endpoints in this type of evaluation, since the cytotoxicity of a substance can be determined on the basis of the increase or reduction in the cell division rate in relation to the control group (Kumar *et al*., 2015; Bianchi *et al*., 2016; Martins *et al*., 2016). According to Gadano *et al.* (2002), if there is a significant reduction in MI, this can be caused by the inhibition of DNA synthesis or blockage in the G2 phase of the cell cycle. According to the same authors, the reduction of up to 22% in MI in relation to the negative control may indicate lethal effects of the substances tested on the test organism. Thus, cultivars with a high MI are suitable for biological assays to assess toxicity at the cytogenetic level when exposed to chemical substances. The cultivars used in this study, Boston Branca, Babá de Verão, Grandes Lagos and Grand Rapids in *L. sativa* and Baia Periforme in *A. cepa* stand out as the cultivars with the highest cell division rates.

According to Bagatini *et al*. (2007), the evaluation of chromosomal abnormalities (CA) is constantly used in cytogenotoxicity tests, which are one of the few direct methods to measure damage in target organisms exposed to toxic substances. The effects of these substances can be detected cytologically by inhibition of the cell cycle, interruption of metaphases and induction of numerical and structural chromosomal changes (Leme & Marin-Morales, 2009). Mutations may also be due to the intrinsic instability of nucleic acids, and the observation of CA as micronuclei, chromosomal bridges, C-metaphases, stickiness and lost chromosome is common, even at low frequencies, in cells not exposed to toxic agents. Thus, in this study, the importance of quantifying CA inherent in each cultivar exposed only to water commonly used as a negative control in bioassays is justified (Aragão *et al.*, 2015; Palmieri *et al.*, 2016). In this study, it was possible to observe that Babá de Verão (Lettuce) and Baia Periforme (Onion) were the cultivars that had the highest frequency of CA, and were among those that presented the highest MI values, and it was possible to suggest that the cell cycle of these genotypes occured irregularly and lacks appropriate studies to understand the reasons for these disorders.

Lactuca sativa L. and *Allium cepa* L. cultivars that obtained the highest average values for the highest number of parameters tested were considered the most suitable for plant assays. Thus, Babá de Verão, Grandes Lagos and Grand Rapids (Lettuce) and Baia Periforme, Crioula and Vale Ouro (Onion) were selected for validation tests with $CdSO_4$, a substance of recognized toxicity in plants (Benavides *et al*., 2005).

Effects of Cadmium toxicity on higher plants have been widely reported in the literature (Bauddh & Singh, 2015; Matraszek *et al*., 2016; Park *et al.*, 2016; Zhu *et al.* 2016). Vieira *et al.* (2015) report that plant exposure to high Cadmium concentrations causes morphological, physiological and biochemical changes. These changes can lead to a marked decline in the absorption capacity and accumulation of essential elements by the roots, mainly due to the general reduction in the metabolism owing to mineral deficiencies, in addition to the reduction in biomass. Similar results were found in this study, in which the toxic action of Cadmium was observed especially for NS, RL, SL, FB and MI.

The exposure of Lettuce cultivars to the metal did not influence %G. However, the germination of Onion cultivars showed to be sensitive to this parameter. Similar results were found by Bavi *et al.* (2011), when studying the effect of Cadmium on *Cicer arietinum*, in which it did not influence plant germination, and Ali *et al.* (2015), also demonstrated that Cadmium significantly reduced the % age of *Brassica nabus*.

Monteiro *et al.* (2009) report the influence of Cadmium on the growth and development of Lettuce, and RL and SL were the endpoints that showed the greatest inhibition. The significant reduction in FB for Lettuce and Onion cultivars exposed to Cadmium corroborate the studies conducted by Aslam *et al.* (2014) in *Capsicum annum* and by Ali *et al.* (2015) in *Brassica nabus*.

Regarding cytogenotoxic effects, the responses found in this study for *Lactuca sativa* show a mean reduction of 92% in cell division when exposed to $CdSO_4$, compared to the negative control (distilled water). According to Gadano *et al.* (2002), if the reduction in MI is higher than 22% of the MI of the negative control, it may indicate lethal effects of the substances tested on the test organism, corroborating the idea that the Lettuce genotypes selected for validation experiments are highly sensitive to toxicity assays.

The results obtained for Baia Periforme and Vale Ouro (Onion) are similar to those presented by Seth *et al.* (2008), in which Cadmium significantly reduced MI in Onion roots, indicating the cytotoxic potential of this metal. *Allium cepa* cv. Crioula is an exception since, in addition to being little mentioned in the literature as a test plant in plant bioassays, it was highly sensitive to $CdSO_4$, once a stimulus in its cell division was observed, when compared to the control, besides the high frequency of CA, with predominance of micronucleus and stickiness.

According to Leme, Marin-Morales (2009), the occurrence of abnormalities in the cell cycle and/or in the chromosomal complement are indicative of the mutagenic action of toxic substances. The highest frequency of micronuclei and stickiness may indicate that $CdSO_4$ has an aneugenic effect, and that both *L. sativa* and *A. cepa* cultivars selected for validation assays were highly sensitive to cytogenotoxicity tests.

Conclusions

The most suitable *Lactuca sativa* L. and *Allium cepa* L. cultivars for phytotoxicity and cytogenotoxicity studies, in descending order of germination, initial growth and cytogenetic parameters, according to statistical analyses (ANOVA and TOPSIS), are Babá de Verão, Grand Rapids and Grandes Lagos (Lettuce) and Baia Periforme, Vale Ouro and Crioula (Onion).

In addition to being the most responsive to the parameters used in ranking, these genotypes were also highly sensitive to characterize the toxicity of a chemical substance.

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