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Performance of tetraploid alfalfa genotypes as exposed to aluminum toxicity

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ABSTRACT

A study was carried out to evaluate the development of 12 tetraploid alfalfa cultivars exposed to Al toxicity in nutrient solution. Newly germinated seedlings of cultivars Alfa 200, Alto, Araucana, Costera, Crioula, Esmeralda, Falcon, F-708, Rio, Romagnola, Valley Plus, and Victoria, were exposed to either 0, 4, 8 or 12 mg·L^{-1} Al^{3+}. Plants were analyzed regarding root length (RL) and dry matter (RDM), aerial part length (APL), and dry matter (APDM), hypocotyl length (HypL) and dry matter (HypDM), epicotyl length (EpiL) and dry matter (EpiDM), and petiole length (PetL), and dry matter (PetDM). Results indicated that, although all genotypes exhibited detectable sensitivity to such a stress, cvs. Crioula, Victoria and Alpha-200 were tolerant to 4 mg·L^{-1} Al^{3+} toxicity. It was also concluded that Al^{3+} levels up to the 4 mg·L^{-1} will be effective for screening tetraploid alfalfa genotypes regarding this type of stress, when evaluations are made in nutrient solution. Finally, RL is the most suitable variable for conducting such evaluations, but all variables related to dry matter in the aerial part are also recommended.

Keywords: Alfalfa; Aluminum Toxicity; Genotypes; Nutrient Solution; Selection

1. INTRODUCTION

It is estimated that 40% of arable land and 70% of non-agricultural soils in the world have a high level of acidity [1]. Such a profile poses a major obstacle to agricultural production due to the direct effects of this abiotic stress on the root system and its consequent impact on plant growth [2-5]. In addition, toxic levels of aluminum (Al) and manganese (Mn), and deficiencies of calcium (Ca), magnesium (Mg) and phosphorus (P) are common in these conditions [3,6].

Aluminum might cause acute toxicity [7-9], thus effecting negative influences on many plant physiological processes [10,11]. Most crops are sensitive to aluminum, including rice, *Oryza sativa*, wheat, *Triticum aestivum* L., corn, *Zea mays* L., soybean *Glycine max* L. Merr., Beans, *Phaseolus vulgaris* L., white clover, *Trifolium repens* L., and alfalfa, *Medicago sativa* L. [12,13]. Some crops, such as buckwheat, *Fagopyrum esculentum* Moench [14], show a high degree of tolerance to aluminum. In addition, there is great genetic variability in response to exposure to aluminum among varieties and genotypes of many crop species [3]. Selection of plants tolerant to aluminum from large groups of genotypes [15-17], and studies of gene action [18,13,19] are the main techniques used to obtain tolerant cultivars.

In most cultivated species, the genetic variability within the germplasm is not pronounced, making it difficult to obtain cultivars tolerant to this stress [16]. In the absence of Al-tolerant genotypes, liming is the alternative to raise soil pH, thereby reducing the toxicity of aluminum [5]. However, liming does not correct the acidity of the subsoil, and this procedure is not always easy to perform and economically viable [20,21]. In the specific case of alfalfa, liming has been recommended only when it is possible to have soil pH increased to at least 6.0 [22,17].

Fuente *et al.* [18] released the first article showing that gene action may increase the tolerance of plants to aluminum. These authors demonstrated the effect of the gene citrate synthase (CS) in *Pesudomonas aerugunosa* by altering the synthesis of citrate in tobacco (*Nicotiana tabacum* L.) and papaya (*Carica papaya* L.), and allowing the selection of aluminum-tolerant strains. Subsequently, other successful attempts explored other genes in other species, such as ALTM1 in corn, sorghum, rice, wheat, rye, barley, and oats [16,23], MATE in sorghum [24], Alt 1, 2, 3 and 4 in rye (*Secale cereale* L.) [25-27], and malate dehydrogenase in alfalfa [20,9]. However, genetic engineering is still an inaccessible alternative to many research groups, either by the relatively great complexity or the high cost, besides posing risks to

environmental and food safety. Therefore, the evaluation and selection of genotypes remain as an important tool in obtaining plant genotypes tolerant to aluminum.

The tetraploid alfalfa (*Medicago sativa* L. ssp. *Sativa* L. & L.) is considered the most important forage legume in the world, with almost 32 million planted hectares mainly in temperate regions [28,29]. In Brazil, alfalfa is being increasingly used in animal production systems, because of its high quality and yield [30]. However, the expansion of alfalfa cropping in tropical areas is hampered by several factors generally associated with climatic and soil limitations. Alfalfa is adapted to soils of neutral pH [31], and this narrow adaptability restricts its use in environments of occurrence of acid or alkaline soils. In fact, soil acidity is reported as a major problem [32], especially by hindering the establishment of symbiotic relations with either indigenous or inoculated rhizobia strains [33,34]. Some evaluation work has been performed to select alfalfa genotypes tolerant to aluminum toxicity [35-38]. The small genetic variability detected in those studies suggests that tolerance to this stress is controlled by a complex multigene system [39,40]. [35,41] used two and four levels of soil pH in the natural environment, and concluded that there was little variation in the response of alfalfa genotypes to Al^{3+} stress. [42], evaluated 192 of 200 germplasm accessions of the USDA alfalfa core collection (PIs) in a natural environment, and concluded that Al tolerance in alfalfa would be very difficult to detect. However, there are reports of some Al-tolerant alfalfa genotypes do exist, as follows: [43] showed that the AT-3 population of their breeding program was significantly more tolerant to aluminum than the AS-3 one. [44] developed a Al-tolerant alfalfa genotype (GA-AT) by selecting materials from pre-existing USA cultivars. [45] identified an alfalfa genotype with increased tolerance to aluminum, and that was later confirmed by [46], using tissue culture approaches. In addition, [47] and [48] detected genetic variability for tolerance to aluminum in regional alfalfa germplasm collections.

It is worth mentioning that most of the above studies were conducted in the natural environment, in field conditions, with few attempts carried out in controlled environments. Although the evaluation of genotypes in artificial conditions do not consider the actual environmental pressure [49,50], field trials are affected by many uncontrolled variables, such as the occurrence of climatic, biotic or nutritional stresses [51], which will be prevented in controlled environments. Besides, significant correlations between the parameters obtained in field trials and those in controlled environments are reported by [37,52,19].

The identification of alfalfa genotypes tolerant to toxic aluminum using hydroponic systems can contribute significantly to studies of variability, function, regulation and gene action, mainly because it is well fit to aid the new molecular procedures [53], besides being used in gene incorporation of in superior cultivars. The incorporation of Al-tolerance genes in high-yielding cultivars may contribute to increase the occupation of acidic soils with alfalfa cropping, especially in sub-tropical regions. The objective of this study was to evaluate the development of 12 tetraploid alfalfa cultivars exposed to Al toxicity in nutrient solution.

2. MATERIALS AND METHODS

Seeds of tetraploid alfalfa cultivars Alfa 200, Alto, Araucana, Costera, Crioula, Esmeralda, Falcon, F-708, Rio, Romagnola, Valley Plus, and Victoria, were sterilized with sodium hypochlorite 2% (v/v) for 10 min and 70% ethanol for 5 min. After washing in distilled water for 3 times, sets of 50 seeds of each genotype were germinated on vermiculite at 22˚C, in a germination chamber without artificial lighting. Four days after germination, 24 uniform seedlings of each cultivar were selected, had the initial root length measured, and immediately transferred to 2 L plastic pots containing aerated nutrient solution. After 24 h, each 12-cultivar seedling group was exposed to fresh aerated nutrient solution, with Al^{3+} levels 0, 4, 8 and 12 $mg \cdot L^{-1}$ added by $AlCl_3$ $6H_2O$ (MW = 241.43). Subsequently, pH levels were adjusted to 4.3, checked twice a day and adjusted with 1 N HCl or 1 N NaOH, whenever needed. Solutions were replaced every other day and regularly brought to volume with distilled water.

Nutrient solution composition consisted of 1.5 mM $Ca(NO_3)_2$, 1 mM K_2HPO_4, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 0.5 mM NH_4NO_3, and minor nutrients (0.32 µM $CuSO_4$, 60.65 µM H_3BO_3, 0.52 µM MoO_3, 11.37 µM $MnCl_2$, and 1.15 µM $ZnSO_4 \cdot 7H_2O$). FeEDTA was added, so as to provide 89.5 mM Fe [54].

Plants were grown for 14 days and subsequently harvested for the evaluations. Manipulations were carried out under controlled conditions (Biotronette Mark III environmental chamber, LAB-LINE Instruments) set at 25 ± 1˚C, 60% RH, 16 h photoperiod and ≈400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR, measured with LI-190SA quantum sensor and LI-189 quantum meter, LI-COR). The experiments were carried out as a ran- domized block design, considering a 12 (cultivars) X 4 (Al levels) factorial, with 6 replications and it was re- peated four times.

Once developed, the plants were harvested and evaluated according to the following: root length (RL), root dry matter (RDM), aerial part length (APL), aerial part dry matter (APDM), hypocotyl length (HypL), hypocotyl dry matter (HypDM), epicotyl length (EpiL), epicotyl

dry matter (EpiDM), petiole length (PetL), and petiole dry matter (PetDM). Data on growth rate (APL) and dry matter (APDM) of the aerial part were obtained by the sum of the respective values obtained with the hypocotyl, epicotyl and petiole 1 and 2. Data on petiole growth rate (PetL) and dry matter (PetDM) were obtained by adding the respective values obtained with petioles 1 and 2. Length evaluations were carried out with a precision ruler and weighing measurements were performed in a Mettler AB-S digital analytical balance (Mettler Toledo).

The data were subjected to the analysis of variance, considering the factors Al^{3+} levels and fixed genotypes, followed by regression adjustment and Spearman correlation analysis among the evaluated variables, according to the methodology proposed by [55]. All tests were performed with SAS statistical software [56].

3. RESULTS AND DISCUSSION

3.1. Physiological Variables as Indicators of Aluminum Tolerance

The analysis of variance (**Table 1**) revealed an interaction between Al^{3+} levels and alfalfa genotypes for the variables root length (RL), aerial part dry matter (APDM), hypocotyl length (HypL) and dry matter (HypDM), and petiole length (PetL) and dry matter (PetDM). Thus, these variables proved to be effective in demonstrating the differences among the studied genotypes when exposed to toxic aluminum in nutrient solution. All variables had a significant effect for the genotype source of variation, demonstrating that there are differences among genotypes regardless of the applied level of toxic aluminum. Moreover, all variables had a significant effect for the factor Al^{3+} level, indicating that the evaluated parameters were affected by the Al^{3+} levels in at least one of the genotypes. The coefficients of variation determined for the variables ranged from 9.33 (APL) to 38.89 (RDM), showing a good accuracy of the estimates that were made in the experiment, possibly due to the efficient environmental control system used for growing the alfalfa seedlings.

Although not all variables yielded interactions between pairs of treatments, the analysis was continued in order to isolate the genotype trait variation as compared to the different toxic aluminum levels. Thus, by applying the linear regression equations, parameters were obtained up to the third degree of the polynomial, for each evaluated genotype. The regression equations and their graphical representations are shown in **Figures 1-4**, in order to demonstrate the isolated effects of the factors genotypes and Al^{3+} levels.

3.2. Growth of Roots and Aerial Part

The trends of the variables relative to growth of roots and aerial part are present in **Figure 1**. Despite a significant genotype × Al^{3+} interaction was detected for the variable RL in the analysis of variance, it is clear that all

Table 1. Summary of the analysis of variance, means and coefficients of variation (CV) for the variables root length, root dry matter, aerial part length; aerial part dry matter; hypocotyl length, hypocotyl dry matter; epicotyl length, epicotyl dry matter; petiole length; petiole dry matter, measured in 12 alfalfa cultivars under stress of 4 levels of Al^{3+} toxicity in a controlled environment.

	Mean Square						
	S.V.	Genotype	Al^{3+}	Genotype × Al^{3+}	Residue		
Variable[1]	D.L.	11	3	33	220	Mean	C.V.
RL		1.18**	1067.55**	0.48*	0.24	2.90	16.91
RDM		0.73**	15.09**	0.18^{ns}	0.14	0.96	38.89
APL		3.74**	362.78**	0.55^{ns}	0.40	6.77	9.33
APDM		0.60**	21.51**	0.095**	0.044	1.21	17.23
HypL		0.19*	46.51**	0.14*	0.090	1.25	24.03
HypDM		0.025**	3.13**	0.0099**	0.0043	0.24	26.87
EpiL		0.38**	3.39**	0.048^{ns}	0.051	2.038	11.04
EpiDM		0.22**	2.60**	0.028^{ns}	0.024	0.68	22.77
PetL		1.42**	108.16**	0.39**	0.18	3.48	12.17
PetDM		0.034**	1.58**	0.0099**	0.0031	0.29	19.50

S.V. = Sources of variation; D.L. = Degree of liberty; Al^{3+} L = Toxic aluminum level; ^{ns} = Not significant, * and ** = Significant at 5% and 1% of probability, respectively by F test. [1]RL: root length; RDM: root dry matter; APL: aerial part length; APDM: aerial part dry matter; HypL: hypocotyl length; HypDM: hypocotyl dry matter; EpiL: epicotyl length; EpiDM: epicotyl dry matter; PetL: petioles length; PetDM: petioles dry matter.

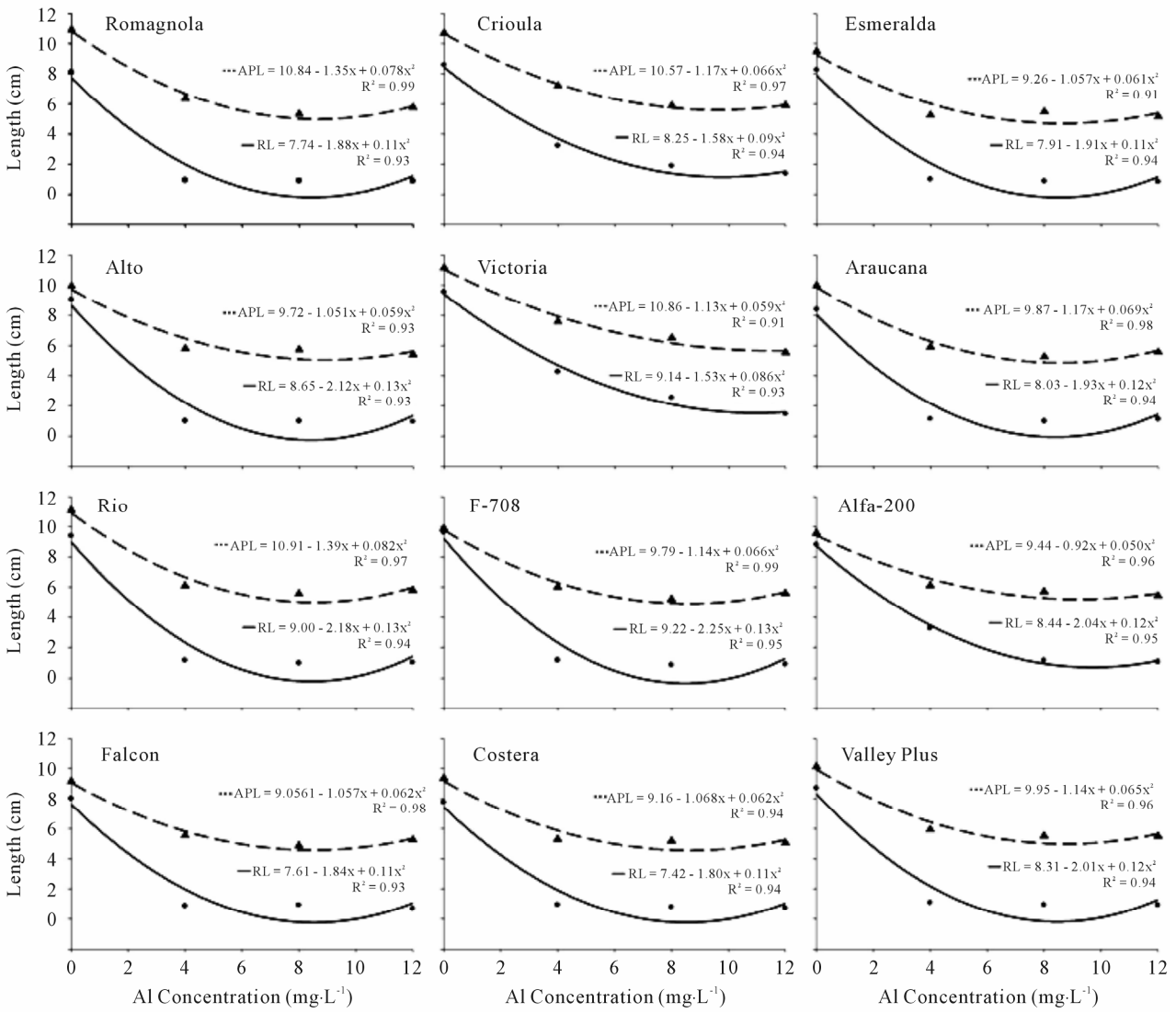

Figure 1. Effect of toxic aluminum levels on root (RL) and aerial part (APL) length of 12 alfalfa genotypes evaluated in a controlled environment.

genotypes exhibited a very similar behavior in response to Al level variation. In a more accurate analysis, it can be viewed, through plotting of the mean values of RL that the greatest differences among genotypes are verified at the lower concentrations of Al (respectively, 0 and 4 mg·L^{-1}). This range of Al level accounted for the detection of detection of the variability found among different cultivars. It can be stated that the levels of 8 and 12 mg·L^{-1} Al were very strong, resulting in very noticeable damage to the RL of all genotypes. Thus, the concentration of 4 mg·L^{-1} Al was the most efficient in discriminating genotypes, as some cultivars showed little reduction in growth at this Al level in relation to the evaluated group of genotypes. Cultivars Crioula, Victoria and Alpha-200 were the least impacted on growth in the level of 4 mg·L^{-1} Al, with RL reductions of 61.6%, 54.5% and 62.5%, respectively. The other cultivars showed very pronounced reductions in the level of 4 mg·L^{-1} Al, with RL absolute values in the same magnitude as those observed at 8 and 12 mg·L^{-1} and mean reductions in root length of nearly 88%. The intercepts (level zero) had relative value range with variations between 7.75 cm (genotype Costera) and 9.6 cm (genotype Victoria) indicating a lack of differences between the materials regardless of the presence of aluminum in the growing solution. These results demonstrate that the cultivar Victoria, besides exhibiting the greatest root growth rate among the evaluated materials, was also the least susceptible to the presence of aluminum in the nutrient solution as revealed by root growth performance, followed by cultivars Crioula and Alpha-200.

When analyzing growth of the aerial part (APL) (**Fig-**

ure 1), the behavior of all genotypes was very similar, confirming the lack of interaction (genotype × Al level) revealed by the analysis of variance. Moreover, data show that the Al-effected stress on growth is much less intense in the aerial part than in the roots, with reductions ranging from 42.7% (genotype Falcon) to 51.0% (genotype Romagnola) under the highest studied Al level (12 $mg \cdot L^{-1}$). According to [12], the main Al damage on plant development is primarily related to cell death of the tissues in direct contact with the toxic element, in this case the roots, which should therefore exhibit more pronounced symptoms of toxicity of this metal. The slower growth of the seedling aerial part occurs due to side effects related to the observed lower root development [38]. By comparing the results on aerial part growth among all genotypes in the absence of Al stress (*i.e.* effect of genotype), it was verified that the genotypes Romagnola, Crioula, Victoria and Rio had the best performance. This indicates that they have a more vigorous initial growth of the aerial part, consequently being recommended as superior genotypes whenever the initial establishment of alfalfa stands is relevant.

3.3. Dry Matter of Roots and Aerial Part

The dry matter accumulation in the roots in all genotypes in response to Al stress is shown in **Figure 2**. The results demonstrate the interaction between the effects of the factors genotype × Al level. It is evident that there are large differences in dry matter accumulation among the genotypes as evaluated both in the response to variations in the applied stress and in the observed intercept values (*i.e.* in the absence of Al addition). Genotypes Valley Plus and Alto, despite having a high content of dry matter in the absence of stress, proved to be very susceptible to Al in the nutrient solution, since their dry matter decrease under such stress was very pronounced (60.2% and 72.4%, respectively). The other genotypes, which apparently suffered minor relative reduction in dry matter content in the roots under the influence of aluminum stress, showed relative reduction values ranging from 55% to 60%, which are still considered very high. Of all genotypes, only Romagnola, Crioula and Coastal had smaller reduction in root dry matter accumulation when exposed to aluminum stress (51.2%, 50.7% and 46.3%, respectively). A higher dry matter content is directly associated with a slower growth of the respective plant tissue, that is, the effect of Al on several physiological processes may result, ultimately, in cell death, and may also be related to inhibition of cell division in a less intense phase, leading directly to lower growth and hence lower gross dry matter content measured in the tissue with slow growth [12]. However, the figure data must be interpreted relatively, taking into account the own genotype performance in response to the addition of Al in solution. Thus, again, it is clear that the alfalfa genotypes studied have high susceptibility to aluminum stress. However, differences in behavior between variable genotypes are sufficient to differentiate their responses to aluminum toxicity.

The dry matter content of the aerial part (**Figure 2**), presented a more uniform behavior and smaller relative magnitude considering the group of evaluated genotypes evaluated. This may have occurred because the effect of toxic Al on the aerial part is secondary, that is, it is an effect due to the primary effect of such stress on the roots, because those tissues are in direct contact with the toxic element. Despite the more uniform behavior, there is variability of DMAP relative to Al stress, as shown by the analysis in **Figure 2**. The genotype Victoria exhibited a more pronounced decrease in comparison to the other genotypes.

3.4. Length and Dry Matter of Petiole, Epicotyl and Hypocotyl

The unfolding of the effect caused by aluminum stress in the aerial part of all genotypes is presented in **Figures 3** and **4**. Those analyses show the effects of growth (L) and dry matter (DM) accumulation (DM) in the subcomponents of the aerial part, namely: petiole (Pet), epicotyl (Epi) and hypocotyl (Hyp). As verified in **Figure 3**, the largest reductions were in petiole growth for all genotypes (**Figure 3**), and the effect was more pronounced from 0 to 4 $mg \cdot L^{-1}$ Al. The greatest proportional decrease in growth was observed in genotypes Romagnola, Rio and F-708, with reductions of 55.6%, 55.4% and 52.0% respectively. The least depressing effect on growth was obtained with genotypes Crioula and Alpha-200, with 41.0% and 44.1%, respectively. These well contrasting differences indicate that this variable is as a good in- dicator for selecting contrasting genotypes in relation to Al stress.

A small reduction was observed in the variable length tent was raised in the nutrient solution. Although visually the changes in hypocotyl length might look similar among genotypes, the analysis of variance (F test) detected significant interaction between genotype and Al level, indicating that the two genotypes differ between each other. A more detailed analysis of the established regressions reveals that cultivars Romagnola, Crioula, Victoria and Alpha-200 were the ones with greater proportionally depressed responses when stressed by increasing levels of Al in the nutrient solution. However, the small magnitude of the observed variation is very difficult be detected and may cause confounding. Therefore, it is not recommended for use as an indicator of alfalfa genotypic tolerance to Al stress.

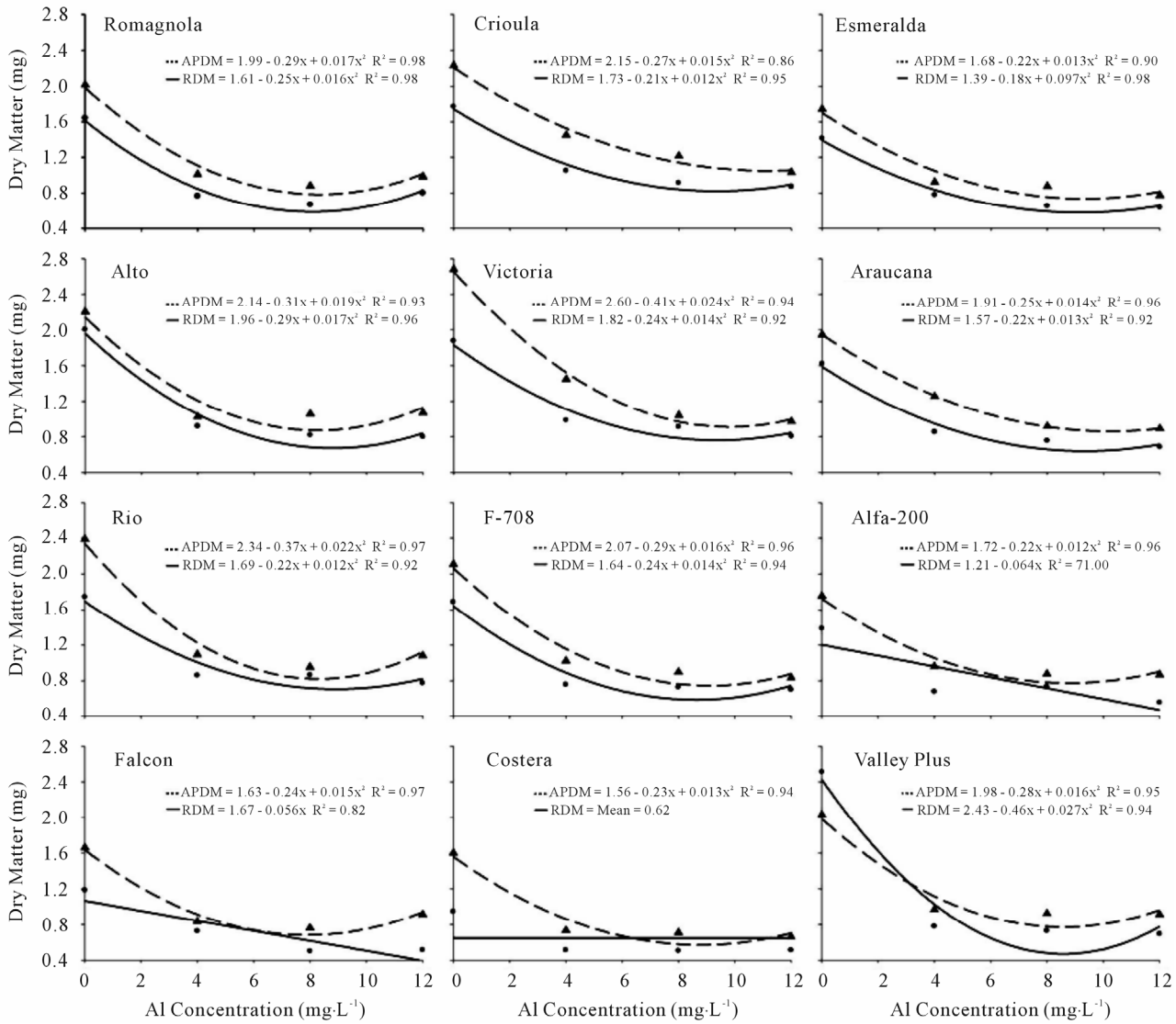

Figure 2. Effect of toxic aluminum levels on root (RDM) and aerial part (APDM) dry meter of 12 alfalfa genotypes evaluated in a controlled environment.

The sub-component of the aerial part epicotyl length was the variable with the lowest variation under exposure to toxic Al. As verified with HypL, epicotyl length is not an adequate measure for screening genotypes against the stress for Al toxicity in nutrient solution, since it masks genotypic responses because of its low susceptibility to stress. Nevertheless, when combining the results of analysis of variance with the established regressions one will find that there was variability among the studied genotypes. Since the variation in EpiL was very small in all genotypes or even zero in the case of genotype Romagnola, one can attribute the occurrence of genotype × Al level interaction specific of this genotype, indicating that it is less Al-susceptible than the others.

With a joint analysis of the variables was performed (**Figure 3**), it was possible to verify that the largest proportion of the response on the aerial part is allocated to the sub-component petiole length, in which the genotypes Crioula and Alpha-200 obtained a marked performance against the stress. It was also noted that the means of the variables EpiL and HypL were very similar in all observed genotypes, suggesting that those are parameters genetically controlled and that their expression is preconditioned, regardless of the occurrence of environmental variations as in the Al stress.

As for the sub-components related to dry matter accumulation in the aerial part (**Figure 4**), it is observed that there were proportionally similar reductions in the three variables (EpiDM, and PetDM HypDM). That is, the evaluation of dry matter of the aerial part should be carried out without total breakdown into sub-components, because none of the evaluated sub-components was noted

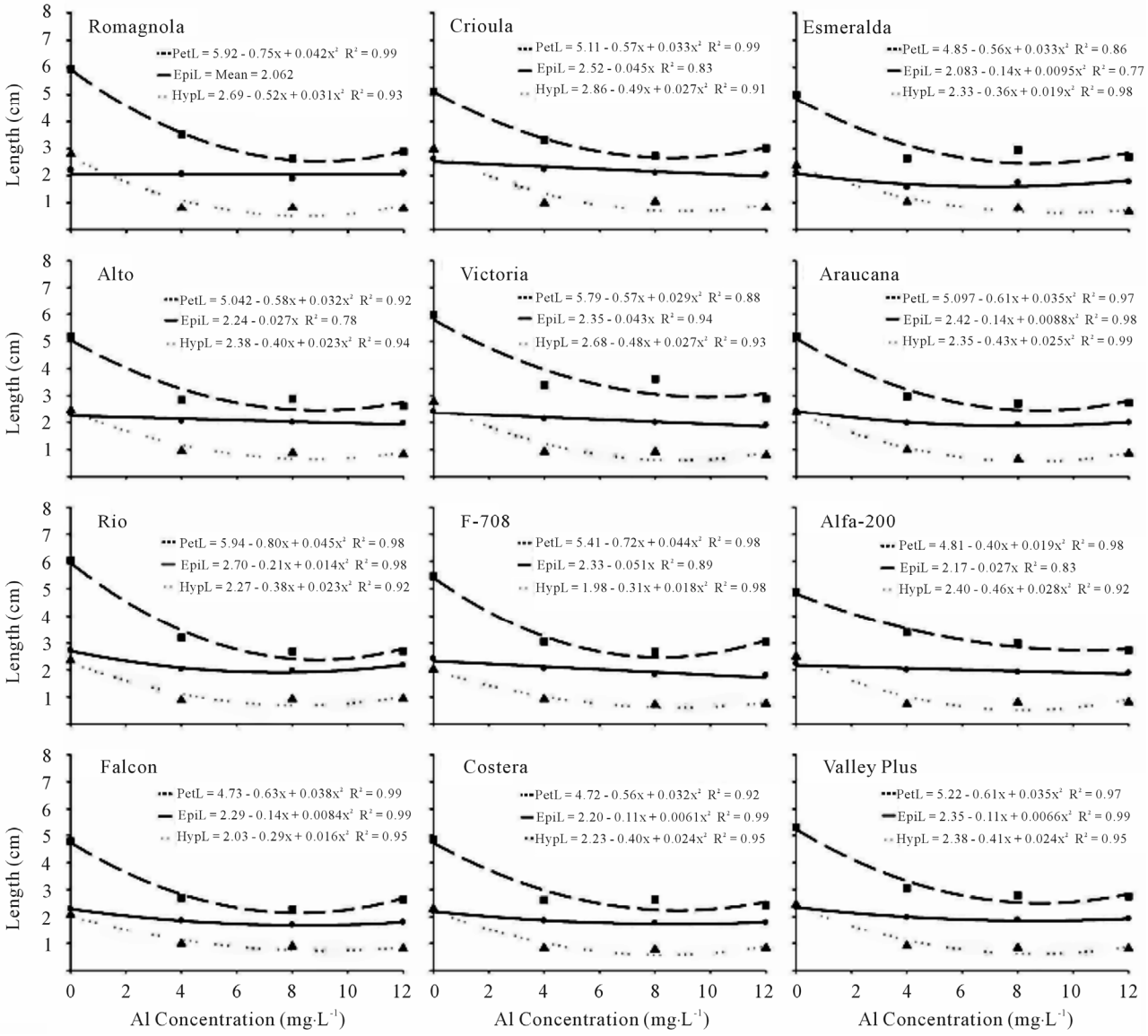

Figure 3. Effect of toxic aluminum levels on petiole (PetL), epicotyl (EpiL), and hypocotyl (HypL) length of 12 alfalfa genotypes evaluated in a controlled environment.

for greater reduction in relation to others, as it was observed in the variables related to growth. Few studies unfold the effects of biotic and abiotic stress on the sub-components of the aerial part alfalfa seedlings [57], so the results of this study have great relevance and introduce a key step for new experimental designs aimed at the early evaluation (seedling stage) using hydroponic systems.

Despite all sub-components of dry matter exhibited remarkable decreases, it can be observed that the dry matter of the epicotyl was the most responsive one. The analysis of this variable indicates that the genotypes Alpha-200, Romagnola and Alto were the most tolerant to Al stress, with proportional reductions of 30.1%, 31.2% and 32.4%, respectively, at the highest Al level (12 $mg \cdot L^{-1}$), and the genotypes Victoria and F-708 the more susceptible ones, with reductions of 55.9% and 48.4%, respectively. The variables petiole and hypocotyl dry matter showed very similar behavior, however, it should be noted that the genotype Falcon had a proportionally smaller decrease of biomass in both the hypocotyl and petioles. On the other hand, the largest proportional reductions in these variables were not clearly detectable among the genotypes.

3.5. Final Remarks

The observed differences in the behavior of genotypes when exposed to Al toxicity in nutrient solution clearly indicate the occurrence of genetic variability for toler-

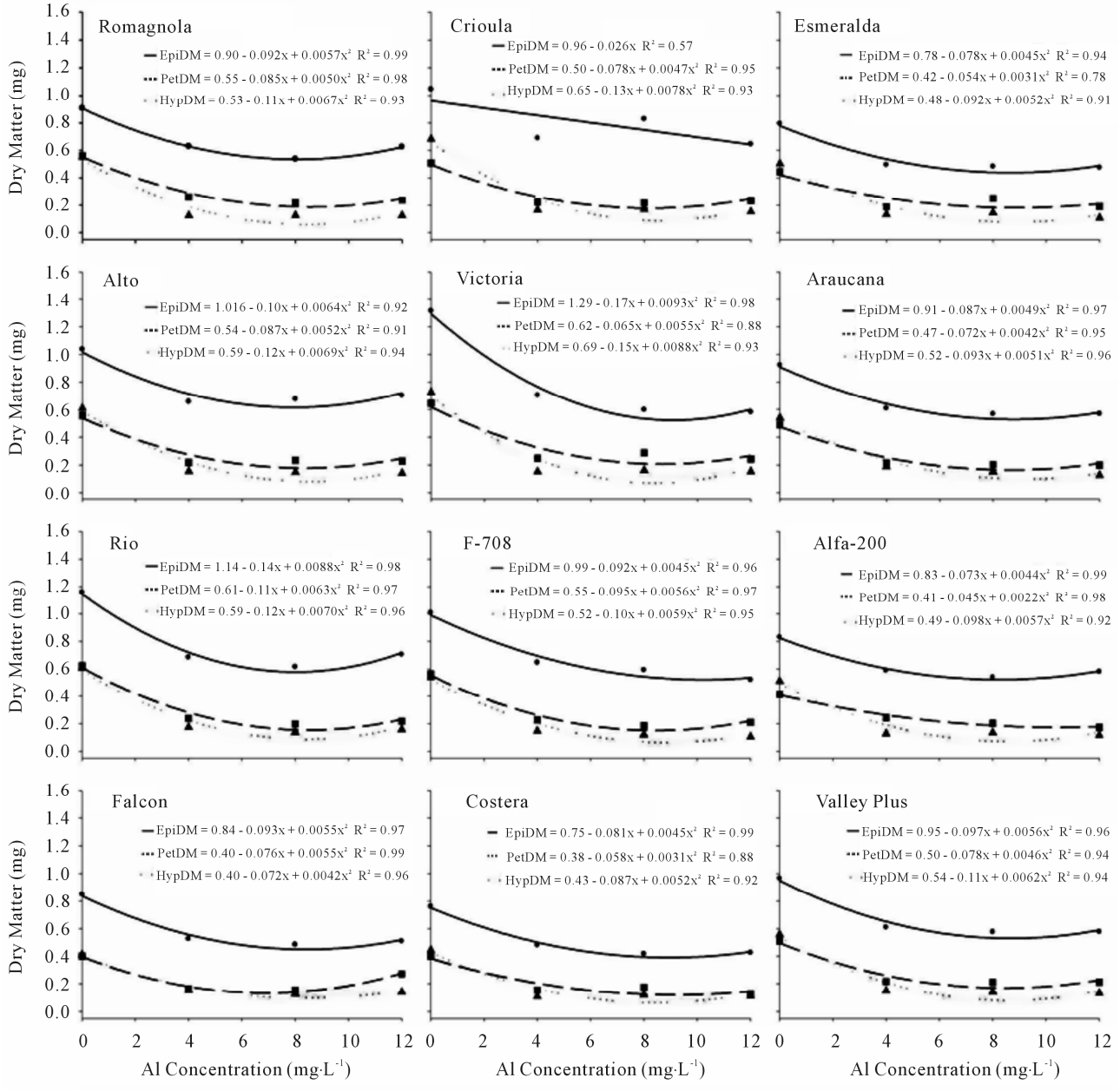

Figure 4. Effect of toxic aluminum levels on petiole (PetDM), epicotyl (EpiDM), and hypocotyl (HypDM) dry meter of 12 alfalfa genotypes evaluated in a controlled environment.

ance and, consequently, the possibility of selection and breeding in order to obtain superior genotypes to be grown in soils with low pH and high aluminum saturation. When conducting a joint analysis of the variables it will be concluded that the genotypes Crioula, Victoria and Alpha-200 suffered minor effects of Al stress on the variables related to plant growth, and genotypes Crioula, Victoria and Araucana the smallest reductions in the accumulation of plant dry matter, at 4 mg·L^{-1} Al in nutrient solution. Thus, these genotypes have potential for use in alfalfa breeding programs aimed at the introgression of genes for aluminum tolerance in the soil. The other genotypes, when evaluated at 4 mg·L^{-1} Al, had the evaluated attributes extremely diminished by the exposure to toxic aluminum and did not differ among themselves in the range of 8 to 12 mg·L^{-1} Al.

The traits in the study were used to compute the phenotypic correlation coefficient with the purpose of evaluating the magnitude and the direction of the influence of one particular trait over another, allowing the degree of association between both to be determined, thus obtaining a trait that is efficient and easy to be measured for further comparison of genotypes. Special care should be taken in order to always estimate the

Table 2. Spearman correlation coefficient for the variables length and dry matter of roots (RL and RDM, respectively), aerial part (APL and APDM, respectively), hypocotyl (HypL and HypDM, respectively), epicotyl (EpiL and EpiDM, respectively), petioles 1 and 2 (PetL and PetDM, respectively), evaluated in 12 alfalfa genotypes exposed to 4 concentrations of toxic Al in a controlled environment.

Variables[1]	RL	RDM	APL	APDM	HypL	HypDM	EpiL	EpiDM	PetL	PetDM
RL		0.6781	0.7517	0.7206	0.6106	0.6187	0.5796	0.6858	0.7378	0.6898
RDM			0.7060	0.7574	0.6263	0.6998	0.5341	0.7010	0.6628	0.6793
APL				0.8394	0.7335	0.7289	0.7055	0.7595	0.9255	0.8192
APDM					0.6470	0.7870	0.7565	0.9398	0.7472	0.8261
HypL						0.8123	0.4277	0.5554	0.5746	0.5806
HypDM							0.4829	0.6647	0.6280	0.6459
EpiL								0.8427	0.5497	0.5292
EpiDM									0.6444	0.6681
PetL										0.8558
PetDM										

Number of observations = 288; All = Significant at 1% error probability by the t test; [1]RL: root length; RDM: root dry matter; APL: aerial part length; APDM: aerial part dry matter; HypL: hypocotyl length; HypDM: hypocotyl dry matter; EpiL: epicotyl length; EpiDM: epicotyl dry matter; PetL: petioles length; PetDM: petioles dry matter.

cause/effect relationships in the analysis of correlation between traits, as indirect effects such as lower weight due to lower growth might lead to confounding in the choice of traits which must end up appropriate in relation to the for purposes of a given experiment [58]. In the present case, these indirect effects are not imposed as a deterrent for the purposes of the experiment, because they are not intended to characterize the effects of stress, but rather compare genotypes against an adverse environmental condition [59]. All traits clearly indicated significant and direct correlation among themselves (**Table 2**). The variable root length is frequently used to evaluate the tolerance to aluminum toxicity in several species, but in many cases the assessment to roots is hampered mainly due to a variety of used substrates [60]. In our study, this variable showed intermediate association with all other evaluated variables. However, our analyses (ANOVA and regressions) also revealed that the variables related to dry matter in the aerial part have desired degree of validity for conducting selection approaches in alfalfa against aluminum toxicity. In fact, they are to be recommended, because they are most easily measured, and also resulted in significant variation and high interaction effect between the factors genotype and Al level. It is worth noting the high association between the variables petiole length and aerial part length (0.9255), and between epicotyl dry matter and aerial part dry matter (0.9398), indicating that the sub-component petiole length is largely related to the total variation of aerial part length and epicotyl dry matter in relation to the variation found in aerial part total dry matter.

4. CONCLUSIONS

Toxic aluminum levels up to 4 $mg \cdot L^{-1}$ are effective for screening studies of alfalfa genotypes in nutrient solution.

The variable root length is the most suitable for screening studies of alfalfa genotypes for tolerance to Al toxicity. The variables related to dry matter in the aerial part are also recommended for such approaches.

All genotypes showed sensitivity to Al toxicity, but the genotypes Crioula, Victoria and Alfa-200 have higher tolerance, up to the level of 4 $mg \cdot L^{-1}$ Al. Hence, they are suggested as primary sources for aluminum tolerance in future alfalfa breeding programs relative to such abiotic stress.

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