RESEARCH ARTICLE

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Assessment of Drought Effect in Cultivars of Perennial Switchgrass (*Panicum virgatum* L.) Based on Elementomic Analysis and Chemometrics

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ABSTRACT

Panicum virgatum L. (switchgrass) is a perennial warm season native grass from North American prairies, which is used as forage due to its adaptability to grow in semi-arid regions. In this work, the assessment of response to drought in two cultivars of contrasting behaviour under water stress - Kanlow and Greenville cultivars- was performed. The aim was to evaluate the behaviour of both Panicum cultivars under water stress conditions based on elementomic study and chemometrics. 50 days old plants were grown in a growth chamber; drought treatment was applied by water suppression until moderate stress was reached. Multi-elemental analysis was performed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Elemental profile data were analyzed by chemometrics methods including Principal Component Analysis (PCA) as unsupervised method and Partial least square discriminant analysis (PLS-DA) as supervised method. Remarkable differences were found based on elementomic study in both cultivars, reinforcing the ability of Kanlow to grown in semi-arid regions, where water and saline limitations are recurrent components. However, the high concentration of Pb found in Greenville samples, shows the ability of this cultivar to be used in the recovering of contaminated soils. Moreover, two elements (Si and Zn) were identified as potential markers to discriminate switchgrass samples belonging to different cultivars or geographic origins. Panicum virgatum cv. Kanlow is a good candidate to incorporate as a forage crop in semi-arid regions; however, cv. Greenville can be more recommendable to be used in the recovery of Pb contaminated soils.

Keywords: Chemometrics, Elementomic, Drought, Panicum virgatum.

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I. Introduction

The elemental analysis techniques have allowed the simultaneous quantification of mineral components present in different plant tissues. This approach to multi-element analysis has revealed the complex regulatory mechanism associated with nutrient balance. Besides, it has made it possible to better understand the complex interactions that occur between the essential components in different parts of plants. The term elementoma was recently introduced to refer to the quantitative and simultaneous study of all elements present in living organisms, regardless of their essentiality and changes in composition in response to genotype and environmental stimuli. In analogy with the nomenclature of omic technologies, such as genomics, proteomics, metabolomics, etc., the elementome gave rise to elementomics as a discipline, such as the ionoma to ionomic [1]-[6]. On the other hand, the multi-elemental content in different organs of plants is considered an indicator of the nutritional status of plants, due to that provides a guide on differential accumulation and transport of minerals according to their physiological status. This aspect includes also an elementomic approach because it focuses on the elements retained in plant tissues [7].

Panicum virgatum (switchgrass) is a perennial species of temperate weather native to the prairies of the United States, which constitutes an important source of food for livestock, but also it is being intensively studied as a source of cellulosic biomass for bioethanol. As forage, P. virgatum produces a dense canopy and an extensive root system, having high efficiency in water and nutrient use. These attributes, in addition to its perennial nature, makes P. virgatum promising for fragile soils in semi-arid and arid regions. In this context, the identification of high-performance P. virgatum cultivars under water stress during early growth is crucial to developing its commercial use and expanding the cultivated area. In previous studies, two P. virgatum cultivars of contrasting behaviour were exposed to moderate drought and re-watering, suggesting a different mechanism for stress response [8]. Under water stress, nutrient absorption is reduced and as a consequence, those cultivars with greater tolerance to drought will have greater mineral content compared to susceptible ones. Likewise, with the purpose of protecting the plant, it has been proposed that there is differential absorption of mineral elements under stress so that the absorption of certain elements in relation to others would be favoured with the purpose of protecting the plant [9]-[13].

The essential nutrients have been classified into two groups based on the amounts required by plants: macronutrients and micronutrients, whose convention has been widely adopted by the scientific community. Both macro and micronutrients play an important role in physiological and biochemical processes in plants such as chlorophyll biosynthesis, photosynthesis, DNA synthesis, proteins modifications and synthesis, redox reactions in the chloroplast and mitochondria, sugar metabolism and fixation of nitrogen among others. In this sense, for example, Zn is a cofactor for more than 300 enzymes and 200 transcription factors associated with the maintenance of membrane integrity, auxin metabolism, and reproduction [14], [15]. However, at high concentrations, some transition elements can produce severe toxicity symptoms in plants, and therefore their absorption and utilization are tightly controlled by plant cells [16], [17]. Thus, toxic elements such as Cd, Cr, Pb and Hg, not only are not essential, they do not comply none physiological functions, but also they are highly toxic at very low concentrations [18], [19].

The mineral elements are necessary for plant growth and play an important role in tolerance to water stress [9]. Micronutrients such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), and nickel (Ni) are important components for plants' better growth and development [10] as well as stress tolerance.

Also, the beneficial effect of other elements such as aluminium (Al), cobalt (Co), sodium (Na), selenium (Se) and silicon (Si) -including their positive influence on plant growth and stress resistance have been documented. These nutrients are involved in increasing plants' resistance to both biotic and abiotic stress, improving survival and maintaining productivity. The possible mechanisms for improving stress tolerance mediated by micronutrients have not been fully explained. In fact, the ability of microelements to mitigate environmental stress can be attributed to several different mechanisms. In the literature, some trace elements have been pointed out as beneficial because comply a functional role during plant growth and productivity under certain conditions [5], [10], [20].

For all the above, this work presents the multi-elemental composition including elementomic and chemometric analysis in two cultivars of Panicum virgatum of contrasting behaviour (Kanlow and Greenville) subjected to drought, through supervised and unsupervised multivariate models.

II. Experimental

A. *Controlled Growth Test*

P. virgatum L. cultivars of contrasting water stress tolerance were used: cv. Kanlow and cv. Greenville. Experiments were conducted in growth chamber located in the greenhouse of the Facultad de Agronomía, Universidad Nacional de La Pampa, La Pampa, Argentina. Plants were grown in pots of 220 cm^3 containing ground substrate (1 part soil and 3 parts sand) in a greenhouse. Ten seeds were sown per pots, proceeding to a partial cleaning 20 days from sowing and the final cleaning 27 days from sowing, leaving one plant per pot.

When plants were 40 day-old, drought treatment was applied by water suppression until moderate stress was reached. Controls plants were grown continuously with normal irrigation. The optimal harvest time was determined when the relative water content (RWC) reached an average of 60% and visual symptoms were observed (drought treatment). The experimental design was completely randomized with 4 replicates per treatment. The aerial part of the plants was collected, and each replicate was a pool of five plants.

B. *Reagents*

All reagents were analytical grade, and all solutions were prepared using distilled-deionized water. Ultra-pure deionized water (resistivity of 18.2 mΩ) was produced by a Millipore ultra-purifier (Darmstadt, Germany). Concentrated HNO_3 (Merck, Darmstadt, Germany) was obtained by distillation into a Berghof suboiling distiller system (Ehingen, Germany).

All the standard solutions for analytical calibration were purchased SCIENCE Plasma CAL, with a 5% (v/v) nitric acid matrix, containing 100 mg L^{-1} of each element. All material used throughout the study was cleaned with 10% HNO_3 for 24 h and rinsed several times with ultra-pure deionized water.

C. *Software and Instrumentation*

All multivariate models (PCA and PLS-DA) were obtained using the The Unscrambler X 10.5 software (Camo, Trondheim, Norway).

Multi-elemental determination was performed by using an inductively coupled plasma atomic emission ICP-AES Varian Vista Pro spectrometer (Palo Alto, California) which includes a glass nebulizer and a cyclonic spray chamber. A Czerny-Turner monochromator with a VistaChip charge coupled device (CCD) array detector was employed in this study. The instrumental conditions and operating parameters such as viewing position and nebulizer pressure were optimized automatically for each analyte with the calibration standard of maximum concentration. An Anton Paar MW 3000 microwave system (Graz, Austria) was used for sample digestion.

Table I shows the figures of merit achieved for the elemental analysis of Panicum virgatum leaves: limit of detection (LOD) ranged from 0.11 to 3.15 µg g^{-1} calculated as 3.3 times de standard deviation of blank signals- limit of quantification (LOQ) ranged from 0.24 to 9.66 µg g^{-1}, relative standard deviation (RSD) and regression coefficients (R^2) obtained from the calibration curve of each element.

D. *Sample Treatment*

After the greenhouse growth was concluded, aerial part of plants was collected and dried in an oven at 60 ºC for three days. To determine the differential absorption of micronutrients, quadruplicate samples were performed according to each treatment: Greenville control (GC), Greenville drought (GD), Kanlow control (KC) and Kanlow drought (KD). Dried leaves were milled in a mortar, weighted and placed in PTFE tubes to be mineralized by a microwave system according to suggested method by manufacturer. Finally, after mineralization samples were diluted to 25 mL with 1% HNO_3 solution and then were analyzed by an ICP-AES spectrometer.

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TABLE I: FIGURES OF MERIT FOUND FOR ANALYZED ELEMENTS IN PANICUM VIRGATUM

Element	LOD ($\mu g\ g^{-1}$)	LOQ ($\mu g\ g^{-1}$)	RSD (%)	R^2
Ag	0.11	0.33	2.21	0.9992
Al	0.91	2.73	0.66	0.9999
As	0.33	0.99	1.23	0.9993
B	1.64	4.92	2.05	0.9994
Ba	1.72	5.16	0.15	0.9997
Cd	0.08	0.24	3.96	0.9996
Cr	0.12	0.36	1.39	0.9999
Cu	0.24	0.72	5.41	0.9993
Fe	0.71	2.13	3.26	0.9988
Hg	1.37	4.11	4.87	0.9992
Li	2.63	7.89	2.62	0.9991
K	3.15	9.45	4.86	0.9989
Mg	3.22	9.66	2.88	0.9998
Mn	1.18	3.54	0.58	0.9990
Mo	0.67	2.01	0.16	0.9994
Na	2.94	8.82	4.49	0.9996
Ni	1.33	3.67	0.74	0.9999
P	1.75	5.25	4.61	0.9992
Pb	3.11	9.33	4.42	0.9991
Si	0.98	2.94	0.68	0.9999
Sr	1.12	3.36	0.86	0.9999
Ti	0.44	1.32	2.21	0.9993
V	0.11	0.33	3.86	0.9994
Zn	1.75	5.25	1.78	0.9998

III. RESULTS AND DISCUSSION

A. Multi-elemental Analysis

Table II summarizes the concentrations of multi-elemental analysis found in leaves of Panicum virgatum cultivars (Kanlow and Greenville) including control and drought treatments. In Table II, the remarkable presence of toxic elements such as As and Pb can be seen. Most of the elements had similar concentrations under drought, compared to the control, in both cultivars. However, marked differences between treatments can be observed in Na, P, Si and Pb. In the case of Na, drought treatment presents less concentration in comparison to control. Opposite that, P presents a significant increase in drought treatment in comparison to control, while Si increases in drought treatment for Kanlow but decreases in the same treatment for Greenville. According to these results, both cultivars accumulate high concentrations of P, while eliminating Na under drought. However, Si shows a differential behaviour between cultivars: under water deficit, while cv. Kanlow increases Si concentration, cv. Greenville decreases it. This behaviour might explain, in part, the ability of Kanlow to tolerate drought. In this sense, the effect of silicon in plants has been widely studied in the literature on other species [21]. In general, Si can be accumulated in concentrations from 1% to 10% of dry matter, which has been attributed to the ability of the roots to absorb this element. Also, Si contributes to minimising water stress because it reduces transpiration by developing a Si gel layer on the epidermal cell wall but makes them hard and resistant, not only to water stress but also to pathogenic agents, increasing the tolerance to diseases. As consequence, the presence of silicon in plants allows a defence against biotic and abiotic agents [20]. The differential increasing of Si in cv. Kanlow, added to the decreasing of Na and increasing of P under water stress might be part of a strategy to overcome drought, being this an adaptative difference between both cultivars.

TABLE II: CONCENTRATIONS OF ELEMENTAL PROFILE FOUND IN PANICUM VIRGATUM CULTIVARS: KANLOW CONTROL (KC), KANLOW DROUGHT (KD); GREENVILLE CONTROL GC AND GREENVILLE DROUGHT (GD) (N=4)

Element	Kanlow		Greenville	
($\mu g\ g^{-1}$)	KC*	KD*	GC*	GD*
Ag	0.41 ± 0.05	0.42 ± 0.07	0.36 ± 0.11	0.38 ± 0.09
Al	23.63 ± 20.28	24.90 ± 5.32	30.72 ± 10.14	24.89 ± 9.45
As	1.23 ± 0.42	1.45 ± 0,32	1.12 ± 0,31	1.69 ± 0.94
B	6.95 ± 2.13	9.63 ± 3.83	7.95 ± 1.27	7.04 ± 2.04
Ba	13.03 ± 0.52	9.87 ± 2.50	10.70 ± 1.74	6.66 ± 2.79
Cd	ND	ND	ND	ND
Cr	0.41 ± 0.11	0.69 ± 0.08	0.42 ± 0.10	0.69 ± 0.32
Cu	8.45 ± 0.21	7.23 ± 0.22	6.87 ± 0.33	7.83 ± 020
Fe	53.92 ± 14.63	53.95 ± 9.55	47.02 ± 10.23	41.79 ± 8.70
Hg	ND	ND	ND	ND
K	<LOQ	<LOQ	<LOQ	<LOQ
Li	15.77 ± 2.82	16.99 ± 10.45	20.32 ± 6.44	14.30 ± 13.40
Mg	26.08 ± 4.02	21.08 ± 0.31	36.11 ± 6.65	21.09 ± 7,01
Mn	22.58 ± 5.09	27.88 ± 7.64	35.52 ± 5.36	21.65 ± 3.29
Mo	3.37 ± 0.85	2.81 ± 0.44	3.56 ± 0.36	3.97 ± 0.58
Na	774.7 ± 137.2	569.5 ± 108.0	790.2 ± 164.9	434.7 ± 155.8
Ni	5.62 ± 1.14	5.55 ± 1.05	4.48 ± 1.24	4.33 ± 0.29
P	<LOQ	709.7 ± 494.0	<LOQ	946.9 ± 170.6
Pb	10.24 ± 5.43	6.82 ± 0.67	38.15 ± 5.06	18.49 ± 2.92
Si	193.8 ± 129.7	283.1 ± 24.6	507.9 ± 94.6	361.9 ± 130.9
Sr	13.30 ± 0.80	12.69 ± 2.69	16.11 ± 1.33	12.41 ± 2.35
Ti	6.46 ± 3.11	1,19 ± 0.40	1.61 ± 0.621	1.28 ± 0.49
V	0.50 ± 0.33	0.41 ± 0.21	0.64 ± 0.21	0.68 ± 0,30
Zn	13.63 ± 2.65	13.42 ± 6.01	6.64 ± 2.38	7.14 1.61

B. Analytical Performance

In order to evaluate the accuracy of analytical results, the analysis of a certified reference material (CRM) was performed: Maize Flour (NCS ZC 73010 Mealie) from National Analysis Center for Iron & Steel (NCS) Testing Technology Co., Ltd. Reference material was prepared using the same conditions than Kanlow and Greenville cultivar samples. Table III compares the obtained concentrations vs the certified values, with recoveries ranged from 89 to 110%.

TABLE III: VALIDATION ASSAY PERFORMED IN CERTIFIED REFERENCE MATERIAL. REFERENCE CERTIFIED MATERIAL: MAIZE FLOUR (NCS ZC 73010 MEALIE) (N=3)

Analyte	Certified value [$\mu g\ g^{-1}$]	Found value [$\mu g\ g^{-1}$]	Recovery %
Cu	0.66 ± 0.08	0.67 ± 0.04	101
K	1290 ± 70	1249 ± 3	97
Mg	180 ± 20	160 ± 6	89
Mn	1.55 ± 0.08	1.52 ± 0.29	98
P	610 ± 30	668 ± 102	110
Zn	2.90 ± 0.30	2.67 ± 0.17	92

C. Multivariate Analysis

As shown in Table II, the multi-elemental data obtained by ICP-AES comprise a matrix of values that generate the so-called first-order data (two-dimensional matrix, columns correspond to element concentrations and rows correspond to treatments and varieties). These data were used to extract relevant information through an unsupervised chemometric technique as principal component analysis (PCA), as well as a supervised method as partial least square discriminant analysis (PLS-DA). These multivariate techniques have been used in classifications, as well as in the search for hidden information, in order to recognize species' geographical origin, ecotypes, qualities and properties, etc. In this work, PCA and PLS-DA were performed using the original variables (elemental concentration) obtaining new variables (latent variables) in order to highlight the relevant information of the system, which is hidden in the original variables. The classification models were obtained using data centered by column to avoid overestimating the variables due to differences in the magnitudes of concentration. Multivariate methods have been widely used to explain certain relationships between data that are difficult to observe, which is useful for identifying hidden differences and establishing relationships between and within data groups [22], [23].

D. Principal Component Analysis

PCA is a multivariate tool that processes an enormous amount of data produced by computers and measurement techniques. PCA was used to search for data trends, by combining the original variables. This multivariate technique provides an initial view of data in a space with a reduced number of dimensions (latent variables or principal components) preserving most of the original variability. PCA focus is to discover the true dimension of data (reducing the number of original variables) and allowing the identification of new latent variables. PCA model was validated by cross-validation method, which means that a sample is left out one time and used to find the validation error. Then, all samples are left out one time and thus, the total error can be obtained [23], [24].

The PCA model explains 93% of the relevant information in the data using two latent variables (Fig. 1a, scores plot). The representation of the interactions between the quantified elements and the experimental conditions tested is also observed: 4 ellipses represent every performed treatment: Kanlow drougth (KD), Kanlow control (KC), Greenville drought (GD) and Greenville control (GC). Kanlow and Greenville are separated by the first latent variable, while Drought and Control treatments are separated by the second latent variable. In Figure 1b (loadings plot) each vector represents the influence of original variables (concentration of elements) on each treatment. The elements Li and P have more influence on KD while Fe and Zn had more contribution on KC. In the case of Greenville, GD has a similar behaviour to KD, where Li and P are two significant variables. However, in control treatments, more differences can be observed between Kanlow and Greenville, because Si was the most important variable for this treatment. Regarding cv. Kanlow, P, Li, Fe, Zn and Al had more influence, while Si, Cu, Sr, Na and even Pb influenced more in cv Grenville. Other elements such as Ni, Cr, Sr, Na, Mo and Mn play a minor role. Regarding elementomic analysis, Na concentration was higher in cv. Greenville is in control condition. The metabolism of this element is relevant for osmotic adjustment under water and saline stress. In relation to P, the drought treatments in both cultivars indicate a higher content under stress, that is, Panicum virgatum optimizes the use of water and maximizes its absorption. The synthesis of photoassimilates depends on the availability of phosphate ion in the cytoplasm and is related to the transport of triose phosphate from chloroplasts to the cytoplasm and to the sucrose synthesis process [25]. During photosynthesis, chloroplasts receive phosphate and release triose-P into the cytoplasm. The flux between carbon and phosphate is mediated by the triose-P transporter, which transports the triose-P/P system through the chloroplast membrane [26]. Furthermore, the partition of assimilates between starch and sucrose depends on an interaction of three factors: the concentration of cytoplasmic phosphate, which regulates the export of triose-P from chloroplasts; the activity of the main sucrose synthesis enzymes; and the regulation of ADP-glucose pyrophosphorylase activity. Therefore, the shortage of phosphate in the cytoplasm limits the synthesis of ATP [27] and the release of triose-P in the chloroplast, which can restrict the synthesis of ribulose-1,5-bisphosphate (RuBP) and consequently limit photosynthesis. Drought periods lasting approximately ten days can drastically reduce the diffusion flux of P from the soil to the plant, causing a significant loss of productivity in plants. The absorption and accumulation of P in plants occur when the soil is well supplied with water, therefore, after rehydration, the absorption and assimilation of P should be efficient as soon as the diffusion of the flow is reestablished. Accordingly, before the loss of turgor and folding of the leaves -both common symptoms observed as a consequence of water deficiency in the soil- there is a loss of productivity due to the low accumulation of P capable to sustain the growth of the plant. There is evidence that P supply could mitigate the damage caused by water stress in the growth and physiology of plants during the C3 cycle [28]. On the other hand, in longer periods of drought (close to 35 days), it is possible that the photosynthetic system acclimatizes, restoring the photosynthesis function. Despite this tendency to maintain the photosynthetic capacity in plants under water stress, the reductions in growth indicate that the photo-assimilates may have been preferentially oriented to the cellular reparation and maintenance processes, reducing the energy available for the plant.

As shown in Table II, Kanlow and Grenville cultivars increase the absorption of P under drought conditions. This could be explained by greater development of the root system, frequent acclimatization in many species under water limitations. Diffusive flux, which is the predominant mechanism of P transport in soil, can limit the P supply of water-deficient plants.

Regarding Si, the concentration of this element was higher in GC than in GD. However, cv. Kanlow showed the opposite: low concentration of Si in control compared to drought treatment, showing a significant difference with Grenville cultivar (Fig. 1b, loadings plot). The performance of Kanlow under environmental restrictions (i.e., drought, salinity, flooding) has been reported by several authors [29]–[34]. Possibly, the high adaptability (that is, good performance in a variety of environmental conditions) demonstrated by this genotype is associated in part with a high mineral concentration. Its large biomass production and its morph-physiological characteristics also position it as an alternative crop for the production of fodder for livestock, the production of bioenergy and the recovery of soils with contamination problems of various kinds. Finally, according to Table II and Fig. 1, it is possible to observe the great ability of cv. Greenville to accumulate Pb in drought and control treatment, exceeding three times to cv. Kanlow, indicates the possibility to use this cultivar as an alternative to remediate contaminated soils by Pb.

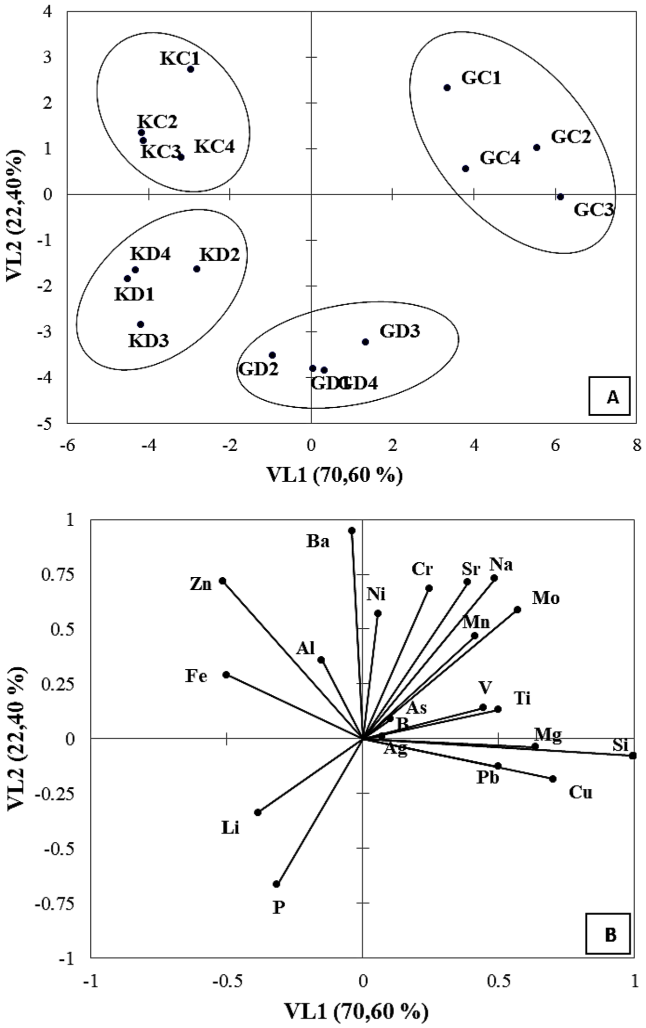

Fig. 1. Principal component analysis (PCA) performed in Panicum virgatum samples (Fig. 1a) and vectors representing the influence of the variables (Fig. 1b).

E. Partial Least Square Discriminant Analysis (PLS-DA)

This supervised pattern recognition technique is a variant of the PLS regression, with the purpose of making qualitative assignation instead of predicting a qualitative parameter. The discriminant analysis by partial least squares is based on the regression model applied to a discrimination context, where the difference lies in the matrix Y (regression concentrations and class discrimination) in order to reduce the dimension of the data and set boundaries between classes. In PLS, the variance of the matrix X (instrumental responses) and Y are decomposed by successive estimates of PLS components that capture the variance and correlation between X and Y. A model is created where the correlation between X and Y is maximized using latent variables (also called PLS components), where the identification of class membership is done through an array of dummy variables (block Y) consisting of ones and zeroes. The constructed PLS-DA model is used to predict new samples, where each class will belong to the samples whose Y values are above the delimited value (threshold) corresponding to that class [35]–[37]. Each sample in the calibration set is assigned a dummy variable as a reference value set as Kanlow drought (KD) = 1000, Kanlow control (KC) = 0100, Greenville drought (GD) = 0010 and Greenville control (GC) = 0001.

The final model was built using internal validation (cross-validation method), where the model leaves out one standard of the calibration set every time. Then, the calibration and validation model error could be calculated through root mean square of calibration (RMSEC= 0.0323) and root mean square of validation (RMSEV= 0.0541) [36], [37]. In this case, three latent variables were selected. The explained variance (cumulative percentage) obtained in the calibration and validation processes with the PLS-DA model, which helps to decide the better number of latent variables. The percentage of explained variance in X and Y blocks were 97.2% and 98.1%, respectively. Fig. 2 shows the score plot of the three latent variables of the PLS-DA model in 3D mode.

According to the classification currently accepted and proposed by Porter [38] the cultivar Kanlow belongs to lowland ecotypes, while cv. Greenville belongs to the highland group. Both groups possess genetic mixtures of different origins and evolution and maintain distinctive phenotypic characteristics. Greenville is a widely adapted highland ecotypes range, from latitude 34°N north to much of eastern Canada and is extremely rare at latitudes below 34°N subject to occasional or frequent droughts. Meanwhile, lowland ecotypes (Kanlow) are found in sites with wet soils prone to seasonality; they are widely adapted in the western portion of the mountain range at approximately 42°N [39]. The lowland ecotype is tetraploid, generally tall, thick and vigorous stems, accelerated growth and bunch type because it has less vigorous rhizomes [40], [41].

Biomass production has a higher yield in places further south, an advantage that decreases with increasing latitude. It yields between 30-50% more than upland cultivars in the transition zone where both are adapted, while in the most extreme northern places, biomass yield is limited by its inability to survive severe winters. In contrast, the highland ecotype Greenville (tetraploid and octoploid) has low growth and thin stems has more vigorous rhizomes and therefore presents a development in the form of grass. This ecotype achieves its greatest development under more moderate conditions of water in the soil, and according to some authors, it has greater tolerance to drought than the ecotype described above, which stands out in humid areas. However, several authors have studied the response of both ecotypes to different stress situations and have found that the lowland ecotype presents greater tolerance to water and saline stress [8], [33], [41], [42]. At present, there is a great diversity of Panicum sp. genetic material, many of them still unclassified according to origin and polymorphisms associated with their habitat. Inbreeding plans, the hybrid cross between lowland and highland materials is a long-sought goal. In this sense, the identification of the genetic pool of origin is crucial to exploit the hybrid vigour of the progeny and the complementarity between the parents. In this context, the identification of markers that indicate the genetic origin of each ecotype is an important contribution to the genetic improvement of Panicum virgatum.

As can be seen in Fig. 2, a dotted straight-line separate both cultivars due to the content of Zn and Si. In addition, the existing separation in four ellipses delimited by the first three latent variables can be clearly observed, which represent each treatment carried out: Kanlow drought (KD), Kanlow control (KC), Greenville drought (GD) and Greenville control (GC), in agreement with the results obtained by principal components analysis. The percentage of discrimination obtained was 100% in all cases and it is shown in the Table IV.

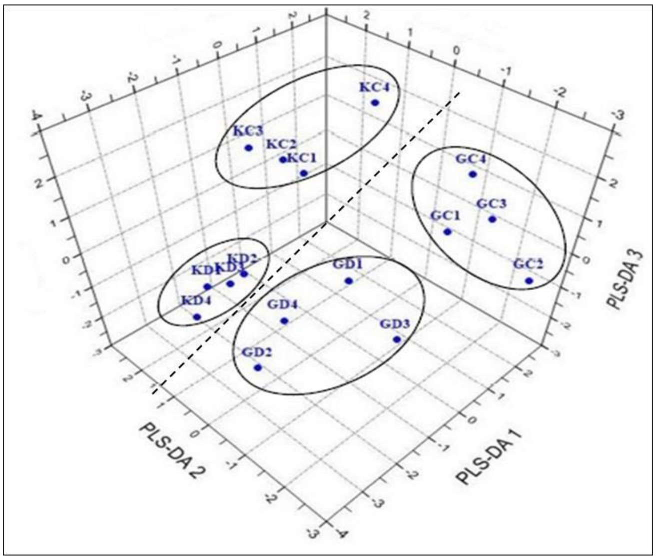

Fig. 2. PLS-DA score plot in 3D for Panicum virgatum samples: Kanlow drought (KD) and Kanlow control (KC), as well as Greenville drought samples (GD) and Greenville control (GC).

TABLE IV: RESULTS OF THE DISCRIMINATION ABILITY OF THE PLS-DA MODEL FOR SAMPLES OF PANICUM VIRGATUM

	% Classification			
	KC	KD	GC	GD
KC	100	0	0	0
KD	0	100	0	0
GC	0	0	100	0
GD	0	0	0	100
Total	100	100	100	100

IV. CONCLUSION

The results showed in this work indicate that Panicum virgatum cv. Kanlow is a good candidate to incorporate as a forage crop in the semi-arid regions. By multivariate analysis, it was observed that in control conditions, Kanlow has a higher concentration of several elements such as Fe and Zn compared to Greenville; these differences can be attributable to the genotype of both cultivars. On the other hand, under drought conditions, the concentration of P and Li increases in both cultivars. The high concentration levels found in Greenville for Pb add new evidence that this cultivar can be suitable to recover contaminated soils with heavy metals by bioremediation. In addition, the Na low level found in both cultivars under drought condition – a frequent strategy in salinity-tolerant species – point to P. virgatum as an alternative to be used in saline soils. Overall, the increase of Si in the treatment of Kanlow under drought as defence mechanism reinforces the concept of the use of this cultivar in the semi-arid regions, where water limitation and saline conditions are usual. Finally, Zn and Si have been identified as potential markers to indicate the genetic origin of Kanlow and Greenville, which is an important contribution to the genetic assessment for P. virgatum. However, more studies could be necessary to assess the ability of P. virgatum to grow in different semiarid environments.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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