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Abstract

Climate changes can influence the genetic diversity of forage plants, which may contribute to the improvement and development of new species. Therefore, this research aimed to evaluate the influence of temperature increase on the genetic diversity of *Macroptilium* accessions based on morphoagronomic descriptors. The experiment was carried out in a growth chamber in a 2×16 factorial arrangement (temperature regimes x *Macroptilium* accessions), with the temperatures consisting of T1 (20–26–33 °C) and T2 (24.8–30.8–37.8 °C) and 16 accessions. Eleven morphoagronomic descriptors allowed estimating the diversity among accessions. The measurements of genetic dissimilarity enabled us to observe the genetic distance between the studied materials, standing out the accessions T1.M3 and T2.S4 as the most divergent (446.01). The morphoagronomic descriptors percentage of leaves and stem diameter were the most efficient for estimating the diversity between accessions. Genetic variability points to the adaptation of *Macroptilium* accessions in the climate change scenario. The accessions more divergent can be explored in genetic breeding programs for the species aiming at the expansion of genetic variability as an adaptation mechanism to heat stress.

Keywords: Animal feed. Climate change. Forage. Genetic variability. Plant breeding.

1. Introduction

Most of the cattle, sheep, and goat herds in the Northeast of Brazil are found in the semi-arid region and contribute to food security, in addition to generating employment and income for families (IBGE 2017). However, the Brazilian Semi-Arid region has adverse environmental conditions, characterized by high temperatures, low water availability, and irregular rainfall, with an average annual precipitation of 464.8 mm (Moura et al. 2019), changing the dynamics of the supply of forage with a direct impact on zootechnical indices and profitability (Voltolini et al. 2019).

These semi-arid environmental conditions could become even more adverse, according to a recent report by the Intergovernmental Panel on Climate Change (IPCC 2021) which estimates an increase of more than 4 °C in the global average temperature by the end of the century, challenging the crop growth and development. In addition, the intensity, duration, and distribution of precipitation are projected to

change, as well as the duration and frequency of extreme weather events (IPCC 2021). This scenario can limit plant growth, as it stimulates leaf senescence, thus reducing biomass (Gray and Brady 2016), which can harm animal feed. Thus, there is a need for forage plants adapted to abiotic stresses.

Plants native to the Brazilian Semi-Arid region have the potential to be used as feed for herds, with many species presenting high nutritional value and forage production, considering the semi-arid environment (Mezzomo et al. 2020). Among them, plants of the genus *Macroptilium* stand out for their adaptation to the edaphoclimatic conditions of the Brazilian Semi-Arid region and contribution to the supply of foods with a considerable protein content for animals (Borges et al. 2018) and nitrogen fixation, improving soil fertility. In addition, this genus has genetic diversity both within and between species, which allows using it in genetic selection and breeding programs for animal feed (Martins et al. 2018).

Plants stressed by the increase in temperature may present changes at physiological and biochemical levels to adapt to the environment. One of the first physiological responses of the plant is the reduction in the stomatal opening, reducing transpiration and, consequently, decreasing the loss of water to the environment (Taiz et al. 2017). Another plant response to high temperatures occurs through the activity of antioxidant enzymes, a plant defense mechanism, which helps in the removal of reactive oxygen species (ROS). These species can damage cells and lead to plant death (Zandalinas et al. 2018). Thus, these physiological and biochemical plant defense mechanisms can contribute to the adaptation of semiarid species, such as *Macroptilium*, in the face of climate change scenarios.

The proper choice of forage species constitutes the starting point for the successful exploitation of livestock farming based on pastures, in which the performance of the forage plant results from its interaction with the environment (Borges et al. 2018). For this selection, genetic variability is a resource in the search for materials tolerant to abiotic stresses (Almeida et al. 2019), contributing to the sustainability of livestock production systems in high lands (Martins et al. 2018).

Some studies have shown that climate changes can influence the genetic diversity of forage plants, such as *Medicago sativa*, *Chloris gayana*, and *Panicum antidotale* (Alhaithloul 2019). According to Pauls et al. (2012), the adaptive potential of plants is associated with the degree of genetic diversity existing among them. The loss of this diversity can be considered a threat, making the species more susceptible to climate changes. Genetic diversity studies under conditions of increasing temperature have not yet been carried out for *Macroptilium*. Air temperature is the climate element that most affects forage species diversity (Sbrissia et al. 2017). Therefore, such studies can support the selection of accessions tolerant to heat stress, mainly for forage grasses, which show different adaptive responses to high temperatures (Al-Soqeer 2016).

Considering the importance of *Macroptilium* for animal feed, the results obtained here may contribute to the selection of accessions more adapted to climate change. It can also contribute to the genetic improvement of the species, guaranteeing better yields. Thus, this research aimed to evaluate the influence of temperature increase on the genetic diversity of *Macroptilium* accessions based on morphoagronomic descriptors.

2. Material and Methods

The experiment was conducted in Fitotron growth chambers, with temperature, light, photoperiod, and humidity control, with dimensions of 4 m long x 3 m wide x 2.50 m high.

Sixteen *Macroptilium* accessions were evaluated, among which seven *M. atropurpureum* (A14, A15, A16, A27, A51, A59, and A9), four *M. lathyroides* (L31, L32, L34, and L7), four *M. martii* (M3, M4, M7, and M8), and one *Macroptilium* (S4). The used seeds were from native species of the Caatinga belonging to the working collection of *Macroptilium* of Embrapa Semi-Arid.

The T1 temperature regime (Table 1) was selected from the minimum, mean, and maximum temperature of the Sub-middle São Francisco Valley region of the last 30 years, and the T2 regime consisted of increasing the temperature by 4.8 °C, as provided for in the report of the Intergovernmental Panel on Climate Change (IPCC 2013).

The experimental design was completely randomized in a 2×16 factorial arrangement (temperature regimes × *Macroptilium* accessions), with three replications.

Seeds from different accessions were distributed in plastic trays containing a commercial substrate. Three seedlings were transplanted into pots with a capacity of 5 L at 15 days after emergence. The irrigation of the pots was conducted on alternate days.

Table 1. Temperature regimes used in the experiment.

Temperature regime	Schedules for each temperature			
	20:00 to 6:00	6:00 to 10:00	10:00 to 15:00	15:00 to 20:00
T1 (20-26-33 °C)	20	26	33	26
T2 (24.8-30.8-37.8 °C)	24.8	30.8	37.8	30.8

T1: Temperature regime 1; T2: Temperature regime 2.

The plants were maintained for 158 days under the tested temperature regimes, and, during this period, three cuts were performed. The first cut occurred 61 days after transplanting, and the main branch length and stem diameter were evaluated using a graduated ruler and a digital caliper, respectively. Leaf and stem portions were separated and submitted to pre-drying in a forced-air circulation oven at 55 °C for 72 hours (AOAC 2000), and subsequent weighing to determine leaf dry mass and stem dry mass. The dry mass data allowed determining the leaf-to-stem ratio, leaf and stem dry matter content, and plant dry mass (sum of the mass of the leaf and stem portions). In addition, the percentages of leaf and stem were determined considering the plant weight and forage accumulation rate (estimated by the total dry mass divided by the number of days).

The second cut was performed 65 days after regrowth and the third cut 32 days after regrowth of the second cut. The same morphoagronomic descriptors mentioned above were evaluated.

The mean data of the morphoagronomic descriptors to quantify the genetic divergence between and within species were used to obtain the distance matrix, using the generalized Mahalanobis (1936) distance. After obtaining the matrix, the accessions were grouped using the mean connection between groups (UPGMA) for the dendrogram elaboration. *Macroptilium* accessions evaluated under the temperature regime of 20–26–33 °C received the abbreviation T1 before the material nomenclature, while the accessions evaluated under the temperature regime of 24.8–30.8–37.8 °C received the abbreviation T2.

Was established the cut-off line on the dendrogram for groups formation. Being the consistency of the groups formed by the hierarchical method was verified using the cophenetic correlation coefficient (CCC). Moreover, the relative contribution of the characteristics to the divergence was determined, using the computer software GENES (Version 2021.1.9) (Cruz 2016).

3. Results

The morphoagronomic descriptors were able to differentiate the accessions when subjected to an increase in temperature and separate the closest accessions (Tables 2 and 3). The diversity of *Macroptilium* accessions was observed for the descriptors percentage of leaves, stem diameter and length, percentage of dry mass; leaf-to-stem ratio, forage accumulation rate, leaf, stem, and plant dry mass, percentage of stem, and percentage of leaf dry mass. The relative contribution of morphoagronomic descriptors to the genetic diversity of accessions showed that the percentage of leaves and stem diameter were the ones that most contributed to the representation of variation between accessions in the environment with a temperature of 24.8–30.8–37.8 °C, contributing with 73.5% (Table 2).

Genetic diversity among *Macroptilium* accessions was also influenced by temperature (Figure 1). Two groups were formed from the dendrogram of the graphical representation of the genetic dissimilarity between *Macroptilium* accessions. The first group was formed by the T2.S4 accession and the second group by the other accessions. The second group generated different subgroups, confirming the existence of genetic diversity among the accessions, influenced by temperature (Figure 1). These results are in common agreement with those found by the dissimilarity measures, as the reliability of the graphical representation of the accessions was 97%.

Genetic dissimilarity between accessions, standing out accessions T1.M3 and T2.S4 as the most divergent (446.01) and accessions T1.A14 and T1.A15 as the most similar (0.77) (Table 3), where T1

represents the temperature regime of 20–26–33 °C and T2 corresponds to the temperature regime of 24.8–30.8–37.8 °C. However, the comparison between individuals belonging to each species within the same temperature regime showed no different behavior between them.

Table 2. Relative contribution of 11 morphoagronomic descriptors used to assess the genetic dissimilarity of *Macroptilium* accessions subjected to the temperature regimes T1 (20–26–33 °C) and T2 (24.8–30.8–37.8 °C).

Variable	T1	T2
Percentage of leaves (%)	0.0	61.7
Stem diameter (mm)	4.8	11.8
Stem length (cm)	0.3	5.2
Percentage of dry mass (%)	0.0	3.0
Leaf-to-stem ratio	0.1	12.5
Forage accumulation rate (g.dia ⁻¹)	0.1	0.0
Leaf dry mass (g)	4.7	0.4
Stem dry mass (g)	0.0	1.6
Percentage of stem (%)	88.5	0.0
Plant dry mass (g)	1.3	3.5
Percentage of leaf dry mass (%)	0.2	0.3
Total	100.00	100.00

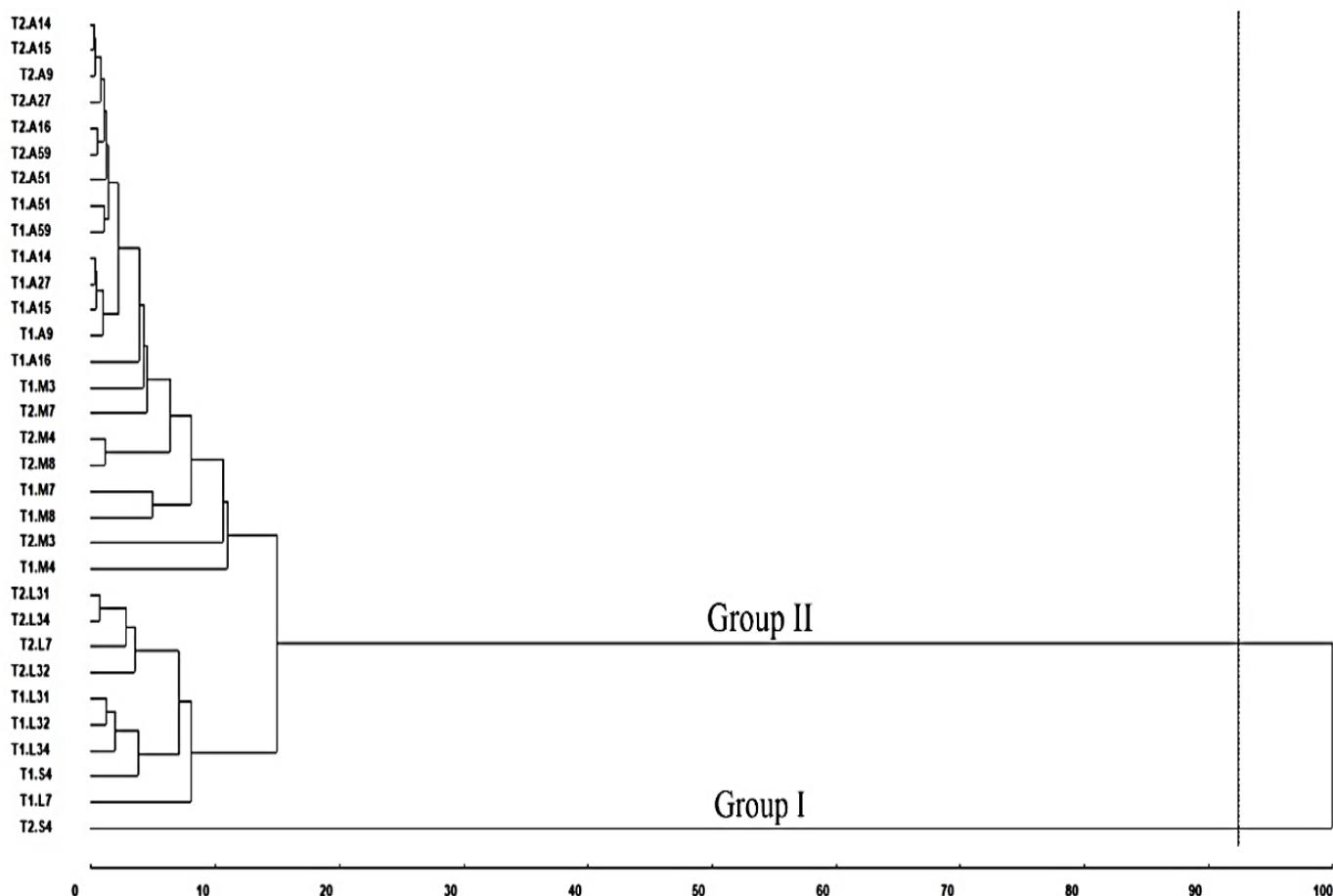


Figure 1 Dendrogram representing the genetic dissimilarity between and within species of the genus *Macroptilium* subjected to the temperature regimes T1 (20–26–33 °C) and T2 (24.8–30.8–37.8 °C), obtained by the UPGMA method, using the Mahalanobis generalized distance, cophenetic correlation coefficient (CCC) = 97%.

A difference was observed only when the accessions were analyzed comparing temperature regimes. Thus, the genetic difference between species due to the increase in temperature was evident, except for the T1.S4 accession, which appeared allocated in the subgroup of representatives of the species

M. lathyroides (Table 3).

Table 3. Measures of genetic dissimilarity between *Macroptilium* accessions subjected to the temperature regimes T1 (20–26–33 °C) and T2 (24.8–30.8–37.8 °C), considering 11 characteristics, based on the generalized Mahalanobis distance.

Accessions	Maximum distance	Minimum distance
T1.A14	2.S4 (387.10)	1.A27 (1.32)
T1.A15	2.S4 (384.25)	2.A14 (8.20)
T1.A16	2.S4 (379.47)	2.A27 (8.19)
T1.A27	2.S4 (383.43)	1.A14 (1.32)
T1.A51	2.S4 (396.77)	2.A9 (2.00)
T1.A59	2.S4 (398.27)	2.A15 (2.00)
T1.A9	2.S4 (384.35)	1.A15 (1.94)
T1.L31	2.S4 (404.03)	1.L32 (4.81)
T1.L32	2.S4 (384.03)	1.L31 (4.81)
T1.L34	2.S4 (399.99)	1.L32 (6.66)
T1.L7	2.S4 (440.58)	2.L34 (14.20)
T1.M3	*2.S4 (446.01)	2.A14 (11.70)
T1.M4	2.S4 (403.39)	1.S4 (25.20)
T1.M7	2.S4 (381.59)	1.S4 (17.99)
T1.M8	2.S4 (341.17)	1.A9 (17.52)
T1.S4	2.S4 (373.07)	1.L32 (9.97)
T2.A14	2.S4 (387.88)	**2.A15 (0.77)
T2.A15	2.S4 (390.19)	2.A14 (0.77)
T2.A16	2.S4 (382.05)	2.A9 (1.72)
T2.A27	2.S4 (380.73)	2.A9 (2.08)
T2.A51	2.S4 (398.02)	2.A9 (3.17)
T2.A59	2.S4 (391.73)	2.A16 (2.09)
T2.A9	2.S4 (387.29)	2.A15 (1.06)
T2.L31	2.S4 (389.07)	2.L34 (2.50)
T2.L32	2.S4 (386.36)	2.L31 (9.34)
T2.L34	2.S4 (400.93)	2.L31 (2.50)
T2.L7	2.S4 (409.35)	2.L31 (10.07)
T2.M3	2.S4 (439.23)	1.A51 (22.33)
T2.M4	2.S4 (420.79)	2.M8 (4.39)
T2.M7	2.S4 (390.69)	2.A27 (10.42)
T2.M8	2.S4 (413.25)	2.M4 (4.39)
T2.S4	1.M3 (446.01)	1.M8 (341.17)

4. Discussion

The morphoagronomic characterization is of fundamental importance in plant breeding programs to estimate genetic variability, contributing to the indication of the best genotypes (Conceição et al. 2014). Thus the perception of variability existing in morphoagronomic traits can contribute to defining the agricultural suitability of genotypes.

Despite the lack of studies on *Macroptilium*, Borges et al. (2018) carried out the morphological characterization of 62 accessions of this legume under current temperature conditions and observed genetic variability among the accessions, with the variables related to the aerial part the ones that contributed the most to the diversity. This result was observed in the present study, reinforcing the importance of aerial part evaluation to help in the discrimination of *Macroptilium* genotypes (Santana Neto et al. 2015). In addition, leaves have higher active metabolism in plants and, consequently, higher concentrations of degradable metabolites, in addition to proteins and nitrogen compounds, being the component with the highest nutritional value, when compared to the stem (Andrzejewska et al. 2020).

In addition to the leaves, stem diameter also contributes to forage quality, as the thicker the stem, the higher the regrowth capacity, thus contributing to increase the number of leaves, which will be used for animal feed (Costa et al. 2004). On the other hand, a thicker stem can indicate higher fiber and lignin accumulations, which can lead to lower plant digestibility.

Thus, considering the relative contribution of morphoagronomic characteristics to diversity, the percentage of leaves and stem diameter were more important (Table 3) when *Macroptilium* accessions were subjected to temperature increase, as recombination between more distant accessions genetically and with better agronomic characteristics enable the inference of the best crosses (Nascimento et al. 2021).

According to Baron and Bélanger (2020), temperature affects the distribution of species, in which temperature changes have a direct effect on the abundance and dynamics of forage species in a region. Al-Soqeer (2016) observed that high temperatures affect the production and quality of forage plants, promoting variability between species, and demonstrated that the genotype x environment interaction can influence forage quality, causing physiological and morphological changes and interfering with plant growth and development (Baron and Bélanger 2020).

One of the first physiological responses of the plant to increased temperature is a reduction in chlorophyll content, photosynthetic activity, and transpiration (Taiz et al. 2017), to prevent water loss. However, this reduction impairs the osmotic adjustment, causing the plant to reduce its ability to tolerate drought, causing losses. In addition, photosynthesis is the primary source of carbohydrates used in plant growth and maintenance (Blum 2017). When exposed to increased temperature, plants, in addition to physiological changes, can also present biochemical changes, such as increased production of reactive oxygen species (ROS). As a defense, plant cells and their organelles (such as chloroplasts, mitochondria, vacuole, cytosol, cell wall, and peroxisomes) employ an antioxidant defense system (Barbosa et al. 2014).

Therefore, in order to tolerate the negative effects of abiotic stresses on plant growth and development, it is necessary to assess how these stresses physiologically and biochemically affect plants (Urban et al. 2017).

In studies carried out with cowpea, a legume with C3 metabolism, as well as *Macroptilium*, Barros et al. (2021) observed that the genotypes most adapted to the increase in temperature were those that showed the least physiological changes, with no reduction in photosynthetic rate, stomatal opening, chlorophyll index, or increase in transpiration, in addition to greater synchrony between enzymes that eliminate oxygen-reactive species.

High temperatures can increase the proportion of the cell wall and reduce the digestibility of both the leaf and the stem in forage plants. It is due to stem elongation, which increases cellulose, hemicellulose, and lignin, and a reduction in the levels of soluble carbohydrates, protein, minerals, and vitamins (Buxton 1996).

According to Soares et al. (2020), the genetic diversity of tropical forages is characterized by variations in their structure and chemical composition. In addition, the genetic range of species due to environmental variation contributes to the selection of plants adapted to a given edaphoclimatic condition, increasing the productive potential (Santos et al. 2011). It allows higher plasticity of the species under adverse conditions (Hoffmann and Sgrò 2011).

The dissimilarity between T2.S4 and T1.M3 is related to the increase in temperature (Table 3), as accessions can present different responses regarding their adaptability to the environment and, therefore, present different growth and productive performance, as observed in studies with cowpea (Matoso et al. 2018; Barros et al. 2021). Moreover, the variability observed in the accessions of the present study evidenced the adaptive response to the increase in temperature.

Borges et al. (2018) evaluated accessions used in this study and observed similar behavior among individuals. However, environmental conditions such as temperature increase were not considered. The inclusion of environmental conditions for the selection of tolerant accessions is important, as the increase in temperature can accelerate the plant phenology, thus changing the growth dynamics of the accessions. Thus, analyzing the response and genetic variation within the population as a function of the environment presents an advance in understanding the adaptation to climate change (Abdel-Mawgood et al. 2005).

Therefore, the diversity between accessions T1.M3 and T2.S4 is an important parameter for genetic breeding, whose recombination can favor genetic diversity between individuals and the generation of a population with a smaller number of homozygous individuals, which can be explored in breeding programs (Cruz et al. 2014).

Plants under stress show biochemical, anatomical, and epigenetic changes that contribute to the

adaptation to the caused disturbance, seeking the conclusion of the biological cycle (Zhu et al. 2017). Furthermore, once subjected to temperature increase, plants create responsive memories that are triggered when exposed again to future adversities, known as “stress mark” (Bruce et al. 2007). In this sense, individuals that carry these memories can be recombined seeking to develop a superior genotype.

The results of this study show different behavior of accessions when subjected to increased temperature, given the existence of genetic variability and the possibility of exploitation within breeding programs, seeking to develop improved and adapted genotypes through the transfer of favorable alleles related to crop tolerance or adaptation to heat stress for *Macroptilium*, enabling intra-or interspecific crosses.

Climate change directly interferes with forage production, the main source of food consumed by animals in the semiarid region (Veloso et al. 2020). In this way, the risks arising from these changes can affect both the production potential of animals and the productivity of forage crops (Angelotti and Giongo 2019). Thus, the prospection of native species, such as those of the genus *Macroptilium*, has the potential to increase forage biomass under conditions of abiotic stress, especially in conditions of high temperatures. In addition, the study of the genetic diversity of native forages tolerant to high temperatures may contribute to plant breeding programs, being an important adaptation measure to increase the resilience of semiarid farmers, promoting regional development.

5. Conclusions

The measurements of genetic dissimilarity enabled us to observe the genetic distance between the studied materials, standing out the accessions T1.M3 and T2.S4 as the most divergent. The morphoagronomic descriptors percentage of leaves and stem diameter were the most efficient for estimating the diversity between accessions. Genetic variability points to the adaptation of *Macroptilium* accessions in the climate change scenario. The accessions more divergent can be explored in genetic breeding programs for the species aiming at the expansion of genetic variability as an adaptation mechanism to heat stress.

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