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ECOPHYSIOLOGICAL RESPONSES OF DIFFERENT PLOIDY LEVELS (TETRAPLOID AND HEXAPLOID), OF *CENCHRUS CILIARIS* TO WATER DEFICIENCY CONDITIONS

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Abstract

Cenchrus ciliaris L. is a perennial grass species that is widely present in Tunisian arid ecosystems. The ability to adapt to water deficiency of two provenances of *C. ciliaris* with different ploidy levels hexaploid (6x) and tetraploid (4x) was studied. Soil Water Content (SWC), predawn leaf water potential (Ψp) and midday leaf water potential (Ψm), number of leaves (NL), and shoot phytomass production were measured. Experiments were carried out under semi-controlled conditions of Sfax region (South of Tunisia). The factors studied were: ploidy level and irrigation treatments. Three irrigation treatments were applied: control T200 (200 mm year^{-1}), T100 (100 mm year^{-1}) and T50 (50 mm year^{-1}). In our experimental conditions, our results showed that *Cenchrus ciliaris* species exposed to different irrigation treatments exhibited a decrease in all measured parameters. Different strategies seem to be developed by both provenances under drought conditions. Therefore, as soil water content decreased, tetraploid (4x) plants showed more negative leaf water potential (Ψp and Ψm) compared to hexaploid (6x) ones. More leaf number as well as shoot phytomass production by 6x provenances, in water deficiency conditions, suggests the ability of these provenances to support low water supply.

Key words: *Cenchrus ciliaris* L., *Poaceae,* Ploidy, Leaf water potential, Leaf growth, Shoot phytomass, Soil water content, Arid bioclimatic.

Introduction

The flora of the North African arid zone of witch Tunisia is a part, is characterized by low species richness (Chaieb *et al.*, 1996; Le Houérou, 2002; Msadek *et al.*, 2021). This particularity is greatly influenced by the climatic and edaphic aridity. In addition to the aridity effects, ecosystems in the southern Tunisia are characterized by high anthropogenic disturbances (over grazing, soil erosion, fragmentation and isolation of plant habitats) accompanied by a decrease in their biological productivity (Morgan *et al.*, 2011; Hussein *et al.*, 2021), and overgrazing, resulting in the degradation of pastoral ecosystems. The decline of grass communities, notably those of perennial ones such as *Stipa lagascae*, *Stipa parviflora*, *Stipagrostis punges*, *C. ciliaris* and *Digitaria nodosa* was reported by Dalhoumi *et al.*, (2017). Ecosystems rehabilitation through plantation and revegetalization prevents phytogenetic resources erosion and facilitates restoration of plant habitats.

Since natural regeneration of plant communities is still very slow in arid regions; therefore, it is necessary to reintroduce artificially, perennial grasses in such areas. Reseeding of species with high pastoral value could be an efficient alternative to restore these ecosystems (Young & Schlesinger, 2015; Ahmad *et al.,* 2020). Under Mediterranean climate, C4 species are known to maintain a functional complementarity with C3 perennial *Poaceae* (Ritchie *et al.*, 2017). Accordingly, these species might be very useful in restoration of degraded ecosystems. *Cenchrus ciliaris* L. is a perennial grass, abundant in the arid and semi-arid areas in the world and has great ecological amplitude able to survive in severe environmental conditions (Maeresera, 2020). It is a C4 grass, expanding up to 1.5 m in height and is known to be a highly livestock forage producer (Atroosh *et al.*, 2018). Now, it is considered as a native species in Africa, Arabian Peninsula, Canary Islands, Indonesia, North India and Pakistan. It is also introduced in Australia, North and South America, Hawaii and Virgin Island (Dhib *et al*., 2014). In Tunisia it appears to be distributed in semi arid and Saharan region as well as in Limestone Mountains associated to their piedmont.

It has long been held that polyploidization is a prominent process deriving in plant evolution (Chen & Huang, 2020). In fact, it was estimated that polyploidy has occurred in the lineage of at least 70% of angiosperms (Yue *et al*., 2006) and in 95% of pteridophytes (Soltis & Soltis, 2000). Compared to their relative diploid level, these latter have developed distinct genetic, anatomical, morphological and physiological features allowing them to exhibit higher tolerance to various abiotic constraints including drought, heat, cold and salt stress, and to colonize large ecological amplitudes and harsher environments (Zhang *et al.*, 2012; Magalhães *et al.*, 2020). In the grass family, *Poaceae* one of the largest families of flowering plants where polyploidy has been well illustrated and the process is still ongoing and widespread. For these reasons, this family has been suggested as a model to study the impact of polyploidy on various aspects such speciation, genome structure, stress tolerance, and gene expression. As *Cenchrus ciliaris* is one of the most important generations of Poaceae family, it constitutes an important component of the major grasslands under tropical and arid bioclimatic (Levy & Feldman, 2002). *Cenchrus ciliaris* was selected because of its root performance in strongly water feeding plant (Hamid *et al.*, 2020); it's highly reproductive elements, its phenological variability and its high adaptability to abiotic stress (Mnif *et al.*, 2003).

In addition, this species is also considered as an excellent candidate because not only for its tolerance to drought and salinity; but also for its good forage qualities (Nawazish *et al*., 2006; Kizima, 2015). In this study, *C.*

ciliaris was selected because of a common perennial grass of Tunisian arid zones on one hand and an important food crop for animals in the pasture on the other hand. Consequently, this comparative study, of various ploidy levels, will result in selecting, the most efficient ploidy level that could be used for the restoration and / or the rehabilitation of pastoral degraded ecosystems.

Our hypothesis: Does polyploidy plant of *Cenchrus ciliaris* can develop diverse responses to water stress under arid bioclimatic? Owing to the high degree of interactions between various factors affecting plant growth, these species could face extinction. Therefore, it is important to evaluate the impact of water scarcity on plant life.

In this work, we tended to assess the effect of water stress on *Cenchrus ciliaris.* A particular attention was paid to plant growth as well as to physiological parameters. The aim of this work is to study the difference between two ploidy levels of *Cenchrus ciliaris* provenances tetraploid and hexaploid, in term of drought tolerance ability.

Materials and Methods

Plant material and culture conditions: In our experimental conditions, polyploid species were selected on the base of phenotypic and genotypic variability that could help the processes of restoration in pastoral degraded ecosystems. Experiments were carried out, inside a greenhouse semi-controlled conditions, in an experimental field located in Sfax region (34° 44’N; 10° 46’E), southern of Tunisia. Tetraploid provenances of *Cenchrus ciliaris* (2n=4x=36) were collected randomly from northern Tunisia, whereas hexaploids of the same species (2n=6x=54) were collected from southern Tunisia (Kharrat *et al.*, 2013). The ploidy level of *C. ciliaris* provenances tetraploid (2n=4x=36) and hexaploid (2n=6x=54) with x = 9 was determined by flow cytometry (Kharrat *et al.*, 2013). Seedlings of tetraploid and hexaploid levels were planted in pots of 10 L capacity. Seeds of *Cenchrus ciliaris* (Table 1) were sown directly in a total of 60 pots, with soil composed of sand (88 %), clay (5%) and silt (7%). After germination, seedlings plants were transferred in pots with one seedling per pot. Tap water irrigation, characterized by a pH of 7.8 and an electrical conductivity of 1.88 mS cm^{-1}, was used during the culture period. Irrigation was done monthly, during one year of growth after seedling step. Each individual, for both genotypes, received annually a total of 200 mm of water equitably distributed (Table 2).

First cutting of one year old grown plant from above the soil surface for each genotype was performed to simulate the zero level of growth during the summer season.

Just after the first cutting plant, three irrigation treatments were applied during six months (Table 3). Water treatment levels were following: Control treatment (T200) corresponding to the average annual precipitation of Sfax region (200 mm.year^{-1}), considered as total watering, T100 corresponding to 50% of the mean annual rainfall (100 mm.year^{-1}), and finally T50 corresponding to 25% of T200 treatment (50 mm.year^{-1}), considered as the average annual precipitation in desert areas, approximately the means annual precipitation for southern Tunisia, based on the work of Anon., (2009) on climate change.

Table 1. Geographical and ecological characteristics of *Cenchrus ciliaris* provenances.

Ploidy level	Tetraploid	Hexaploid
Code	4x	6x
Geographic location	North of Tunisia	South of Tunisia
Latitude	36°73’N	33°73’E
Longitude	10°24’E	10°28’E
Climate Type (Emberger,1955)	Semi arid	Lower arid
Winter	Cold (7-16°C)	Mild (12-20°C)
Summer	Hot (20-30°C)	Dry and Hot (30-35°)
Mean annual rainfall (mm)	500-600	100-200
Soil type	Clay and Loam	Sand and Limestone

Table 2. Frequency and quantity of irrigation applied for Establishment step.

	Seedling provenances (July 1st 2012) and Establishment Step											
	Quantity of irrigation (mm) 2012						Quantity of irrigation (mm) 2013					
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
4x	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65
6x	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65	16.65

Table 3. Water stress application in growth period 2013.

	Water stress application 2013					
	July	Aug.	Sept.	Oct.	Nov.	Dec.
T200 (mm)	33.33	33.33	33.33	33.33	33.33	33.33
T100 (mm)	16.65	16.65	16.65	16.65	16.65	16.65
T50 (mm)	8.10	8.10	8.10	8.10	8.10	8.10

Measured parameters: Series of parameters were measured along the experimental period. The plant growth phenology, in terms of number of leaves, was observed. However, given the large number of leaves formed during the short observation period, a growth unity (GU) represented by the structure established by the stem during an uninterrupted lengthening phase was considered (Chaieb *et al.*, 1996). In addition, physiological parameters as predawn leaf water potential (Ψp) and the midday leaf water potential (Ψm) were measured at 5 am and 2 pm respectively by using the model 1000 pressure chamber instrument (USA) with ten leaves per treatment according to Scholander *et al.*, (1965). Whereas, soil water content (SWC) was determined at 9 am with a WET sensor user manual v1.3 (UK) every 2 weeks.

At the end of experimental period, a ground level cut, was performed again, the phytomass obtained is dried in an oven at 75 °C during 48 hours, and weighed, in order to know the effect of water treatment on the phytomass.

Statistical analysis

Multivariate analysis of variance MANOVA function (by SPSS 22) was performed to test the effects of water stress, provenances and date of measurement on all the variables studied.

Results

Water potential of *Cenchrus ciliaris*: Results of multivariate analysis of variance (MANOVA) were shown in Table 4. The effect of treatment, provenances and month on Ψp showed a significant difference between all the studied parameters. In terms of Ψp, Table 4 exhibited an interaction between two factors (provenance versus treatment), while Ψm manifested interaction between two factors (treatment versus month). Soil water content (SWC) and number of leaves (NL) showed interaction between three factors (provenance versus treatment versus month). Finally, the shoot phytomass indicated interaction between two factors (provenance versus month).

Leaf water potential values, in both provenances, were lower in stressed treatments in comparison to control ones T200, while hexaploid (T50) developed low values of leaf water potential respectively Ψp (-1.70 MPa) and Ψm (-3.2 MPa) (Fig. 1A). However, tetraploid genotype, (T50) showed leaf water potential values varying between -2 MPa and -3.8 MPa respectively for Ψp and Ψm **(**Fig. 1B). Tetraploid provenance showed more negative values of leaf water potential when comparing with hexaploid. In both ploidy levels, soil water content showed 20-45% at the beginning of drought period, compared with 5-40% at the end of drought period (Fig. 2), while the soil water content dropped to below 5% in stressed treatment T50. It was decreased by 50% compared to control treatments. In this case, 75% reduction in water regime was associated with 50% reduction in soil water content.

Table 4. Results of multivariate analysis of variance (MANOVA) performed for parameters.

		Ψp	**Ψm**	**SWC**	**NL**	**Phytomass**
Provenance	ddl	1	1	1	1	1
	F	5,249	62,437	192,104	4,82	80,436
	Sig	0,023	0	0	0,029	0
Tr	ddl	2	2	2	2	2
	F	70,436	78,132	328,15	550,597	369,157
	Sig	0	0	0	0	0
Month	ddl	5	5	5	5	2
	F	0,956	12,226	344,906	184,559	41,319
	Sig	0,445	0	0	0	0
Provenance × Tr	ddl	2	2	2	2	2
	F	10,194	2,78	1,629	0,032	3,652
	Sig	0	0,064	0,198	0,968	0,028
Provenance × Month	ddl	5	5	5	5	2
	F	1,538	0,572	11,75	6,7	7,523
	Sig	0,177	0,722	0	0	0,001
Tr × Month	ddl	10	10	10	10	4
	F	2,635	5,623	15,87	25,69	2,103
	Sig	0,004	0	0	0	0,083
Provenance × Tr × Month	ddl	10	10	10	10	4
	F	1,781	0,37	9,404	4,383	1,154
	Sig	0,063	0,959	0	0	0,333

*Significant difference ($p \leq 0.05$)

Provenances: Hexaploid and Tetraploid; Tr: Treatment water; Month: Month of measurement: Provenances (n=2); Treatment (n=3); Month (n=6).

ψp: Predawn leaf water potential; ψm: Midday leaf water potential; SWC: Soil water content; NL: Number of leaves; Phytomass

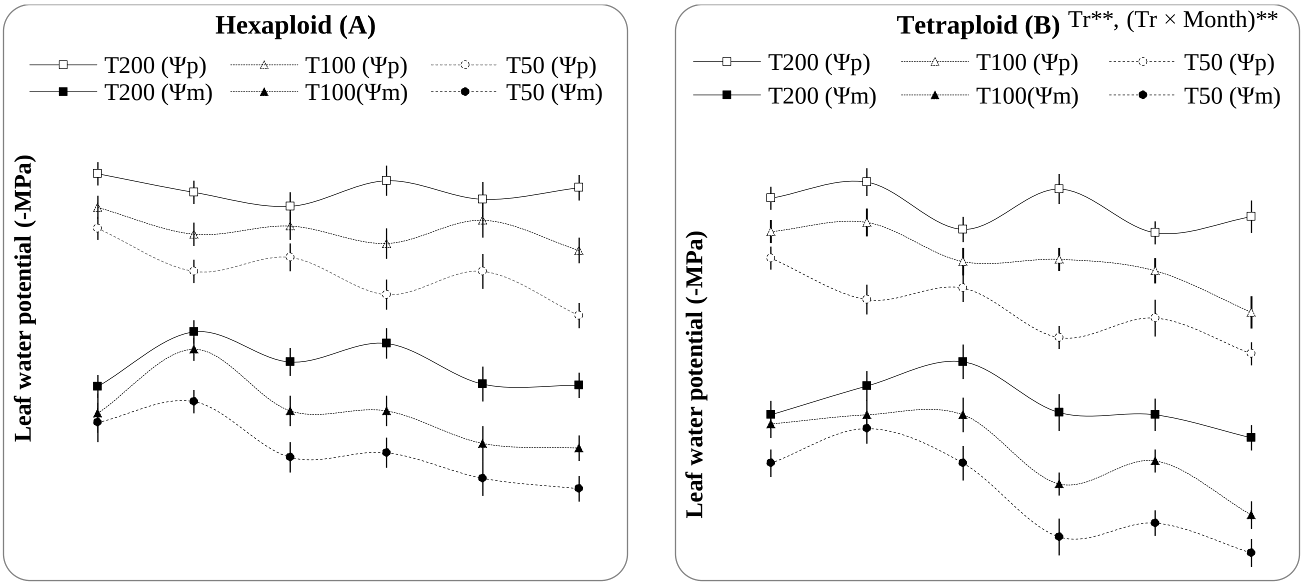

Fig.1. Leaf water potential, of the both provenances, under water stress. The bars are the average of ten replicates (±SE).
**: Significant difference at ($p \leq 0.01$).

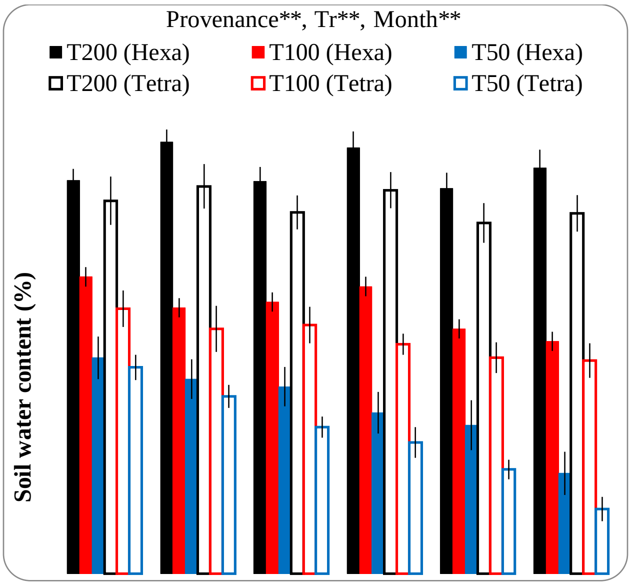

Fig. 2. Soil water content (SWC) under water stress for the both provenances Hexaploid and Tetraploid. The bars are the average of ten replicates (±SE).
**: Significant difference at ($p \leq 0.01$).

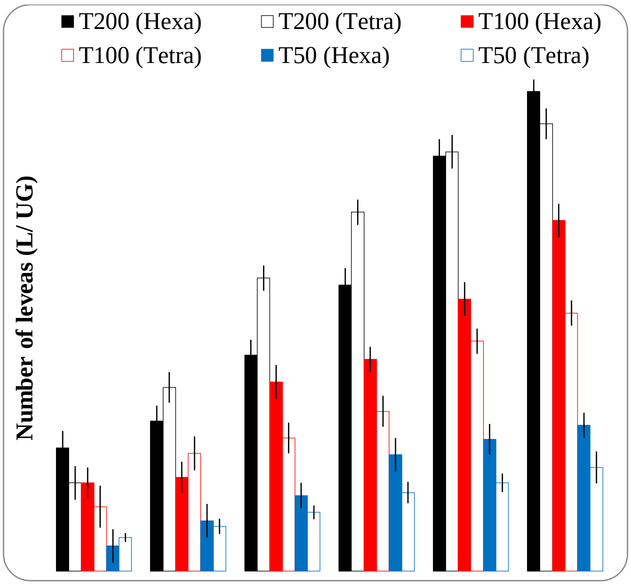

Fig. 3. Leaf number under water stress for the both provenances Hexaploid and Tetraploid. The bars are the average of ten replicates (±SE). *: Significant difference at ($p \leq 0.05$); **: Significant difference at ($p \leq 0.01$).

Growth phenology: A total mean of 9 leaves per unit of growth (L/UG) was observed, in both ploidy levels, from the starting of the experiment and a total mean of 40 leaves per unit of growth was shown at the end of drought (Fig. 3). In comparison with different treatments, T50 showed the least number of leaves. In addition, plant mortality has occurred at the end of the experiment. For both stressed treatments, (T100 and T50), initiation of new leaves as well as growth were slowed down and finally stopped.

In comparison with tetraploid provenances of *Cenchrus ciliaris*, hexaploid ones displayed an intense vegetative growth (number of leaves) and morphological variation of leaves. In fact, hexaploid individus are characterized by small and narrow leaves. For both stressed treatments, polyploidy plants have shown not only resistance to drought but also a different defense mechanism, such as decrease in leaf number. For both treatments (T100 and T50), the aboveground phytomass measurements, during two different periods (July 2013 and January 2014), showed a big difference (Fig. 4).

For 4x and 6x provenances, the shoot phytomass production was reduced after six months, under water stress. In addition, T 50 has shown higher reduction in leaf phytomass. As a case in point, hexaploid genotypes decreased from 12.17 to 7.77 g per tuft, at the end of water stress period, whereas for tetraploid provenances, it decreased from 10.11 to 4.61 g per tuft of *Cenchrus ciliaris* L. However, at the same period, the shoot phytomass showed more than 70 % reduction during six month of water stress.

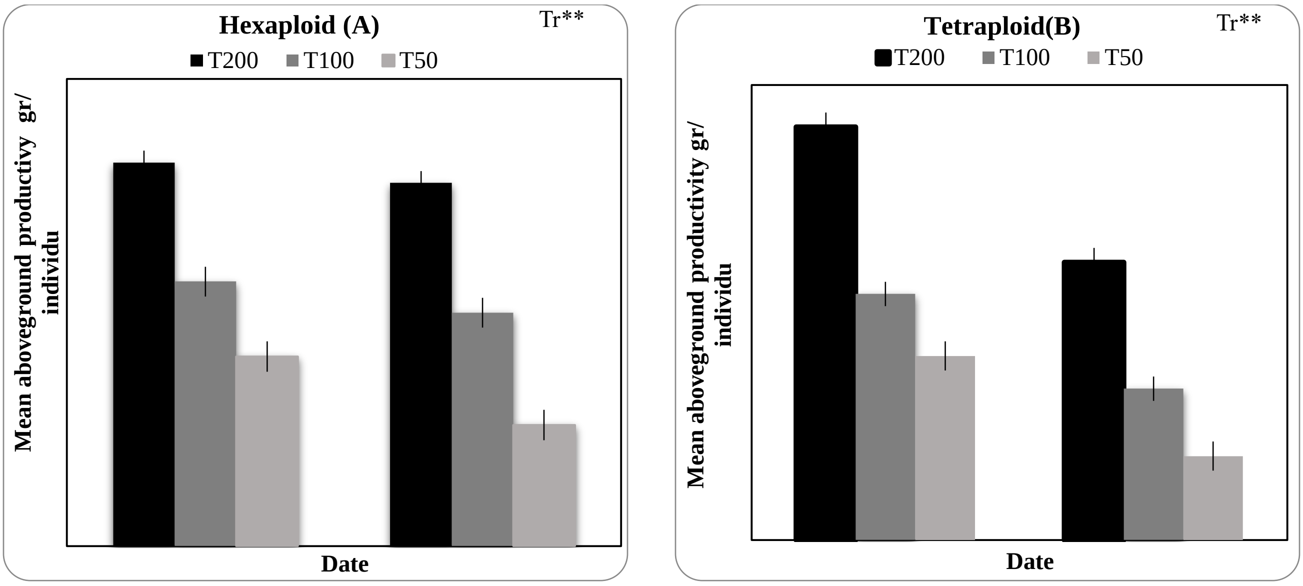

Fig. 4. Means aboveground productivity for both provenances Hexaploid (A) and Tetraploid (B). The bars are the average of ten replicates (±SE). **: Significant difference at (p≤0.01).

Discussions

Cenchrus ciliaris responses to water stress are variables and diverse, depending on the studied physiological parameter (Marshal *et al.*, 2012). This species occupies diverse large range of climates; it can tolerate a mean rainfall varying from 200 to 2670 mm (Marshal *et al.,* 2012). *Cenchrus ciliaris* is known to be adapted to arid climates and dry-land agricultural ecosystems (Amari *et al.,* 2017). It is well known that under adequate soil moisture conditions, plant species will have an intense vegetative growth and consequently producing an important phytomass. Nevertheless, plants tend to develop defense mechanisms allowing them to survive under water deficiency conditions (Dhib et *al.*, 2014). Among mechanisms allowing the plant to increase its tolerance, the reduction of its leaf water potential is a common way maintaining cell function through elevated soil water content (Dhib *et al.,* 2014). In the present study, our results showed a significant decrease in both Ψp and Ψm (Fig. 1) as well as in soil water content (Fig. 2). The ability of *Cenchrus ciliaris* to keep its relative water content at an appropriate level can be considered as a strategy developed in order to avoid dryness in its tissues (Marshall *et al.,* 2012). It was assumed that osmotic adjustment is responsible for maintaining an adequate SWC in plants (Yooyongwech *et al.,* 2016). Our results are consistent with other works showing that keeping water status under drought, with several physiological traits, could be linked to water balance (Martínez–Vilalta *et al.,* 2014). Kharrat *et al.,* (2014) thus suggesting that the two ploidy levels have responded significantly to minimum irrigation. In these conditions, low SWC seems to be behind an increase in leaf water potential, which in turn, leading to a high water demand thus indicating the role of the offset between SWC and ΨL in plant water supply. In this context, the soil water seems to be an important factor influencing water status in arid environment (Ali *et al.,* 2021).

According to Andjelkovic (2018), water stress seems to be a very important limiting constraint in plant growth and development. It greatly suppresses both elongation and expansion growth. In our conditions, water deficit negatively affected the plant growth in both the C. *ciliaris* provenances (Fig. 3).

In our experimental conditions, phytomass productivity of shoots was significantly reduced in response to water stress, with phytomass being more impacted in T50 than in T100 (Fig. 4). Our results were similar to those of Hussain *et al.,* (2015) who reported a decrease in leaf number as well as in plant shoot phytomass production under water stress.

Our results suggest that plant tolerance to drought appears to be controlled by several physiological and biochemical parameters. The study of the correlations, between the different parameters, revealed that factors as (ψp) and (ψm) as well as, shoot phytomass, NL and SWC, were positively correlated. Under these conditions, the low correlation between SWC and NL parameter appeared to be related to a series of factors generated under water stress (Lazaridou & Koutroubas, 2004). Our results showed that in water stress conditions, both growth and phytomass production seemed to be affected. According to Kharrat *et al.,* (2013), when climatic conditions change from semi arid to arid, *Cenchrus cilaris* tends to maintain its growth by keeping its water content at an adequate level ensuring the progress of its metabolic activities. Individuals of (6x) showed a better strategy keeping its growth more important than that of 4x ones. In these conditions, genotype responses variability to drought appears to be influenced by chromosome number.

Conclusion

Two ploidy levels of a buffel grass *(Cenchrus ciliaris* L.) were investigated over six month of water stress using three irrigation levels. As a result, the two ploidy levels have demonstrated several adaptive strategies that can promote its survival and development under water stress conditions. Our findings have confirmed that physiological and physiogenic characteristics of *C. ciliaris* plants are both behind the great ability of this species to remain green and its ecological predominance in hot and arid environments. In our experimental conditions, the comparative analysis between two ploidy levels of *C. cilairis* revealed that hexaploid individuals appear to be the most successful candidate for use in a future ecosystem restoration program.

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