



## Environmental variation and geographic distribution of the *Brachypodium distachyon* grass complex species' in Tunisia.

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### Abstract

The *Brachypodium* genus contains the model grasses *B. distachyon*, *B. stacei* and *B. hybridum*, that are useful for molecular and physiological studies relevant to grain, pasture and bioenergy crops, as well as ecology. In this paper, we analyze the natural variation in climate/geographical diversity that exists within these species in the Tunisian territory. To capture the genetic diversity within this species complex in relation to their geographic distribution in Tunisia, 360 of *Brachypodium* accessions from the Tunisian territory were collected and genotyped by ALB165 marker. Overall, 314 samples were classified into *hybridum* and the rest as diploid for one of the two progenitor's species (*B. distachyon* and *B. stacei*). Environmental niche analysis indicated that, generally, *B. distachyon* grows in higher, cooler, and wetter places north of 36°; *B. stacei* in lower, warmer, and drier places; and *B. hybridum* in places with intermediate ecological features and across latitudinal boundaries but also overlapping with those of its parents, more often with those of *B. stacei*.

## 1. INTRODUCTION

Understanding climatic breadth within species can inform growers about what crop varieties are suitable for their locations and can also aid seed collectors about new locations for future collections. *Brachypodium* native range is the Mediterranean region, with accessions commonly found through southern Europe, North Africa and Eurasia. However, worldwide herbarium records show *Brachypodium* is now present on all six continents except Antarctica (Garvin et al. 2008). This wide geographic range and climate tolerance in both the native and introduced range, as well as a key evolutionary position, mean *Brachypodium* is a rich resource of allelic diversity. The *Brachypodium distachyon* complex includes three annual species: *Brachypodium distachyon* ( $2x = 10$ ), *Brachypodium stacei* ( $2x = 20$ ) and *Brachypodium hybridum* ( $2x = 30$ ). The three

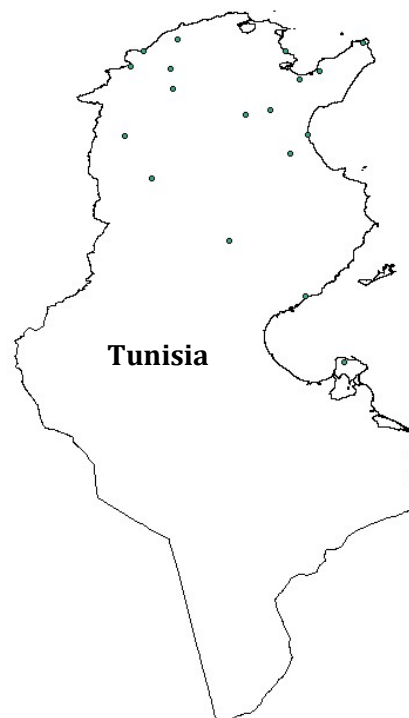
species in this complex are difficult to distinguish in the field and include the diploid *B. distachyon*, the diploid *B. stacei*, and the allotetraploid *B. hybridum*, which contains one *B. distachyon*-like genome and one *B. stacei*-like genome (Hasterok et al. 2004; Catalán et al. 2012; Idziak et al. 2014). To add to the complexity, there is evidence of distinct subgroups of *B. distachyon* (Hasterok et al. 2004; Catalán et al. 2012; Idziak et al. 2014; Tyler et al. 2016). The three species of the complex show a native geographic distribution that covers the entire circum-Mediterranean zone, (Schippmann, 1991; Garvin et al., 2008; Catalán et al., 2012). They grow in different environments, latitudes, and elevations, representing a wide range of biotic and abiotic conditions that may be associated with adaptive natural genetic variation (Garvin et al., 2008; Manzaneda et al., 2012). However, the specific distribution of each of the three species of the

complex has still not been determined due to the strong morphological resemblance between the three species. When overall climate is found to be associated with genomic variation, it is of interest to investigate which particular environmental variables are the drivers of natural selection. This can be done by clustering environmental variables across geographic locations. In this context, we aimed to analyze the natural variation in climate/geography diversity that exists within these species in the Tunisian territory.

## 2. MATERIALS AND METHODS

### 2.1 Plant material and growth conditions

In total, 360 accessions belonging to eighteen populations of *Brachypodium* complex (*B. distachyon*, *B. stacei* and *B. hybridum*) representing all the bioclimatic stages and covering the geographic range of the species in Tunisia were used in the current study (Fig. 1.). Since it was difficult to discriminate morphologically between the three species, seeds of 20 mother plants within each location were collected randomly and at widely spaced intervals without a priori concerning their specific rank. Four seeds from each accession were sown in plastic pots filled with 5 kg of sandy soil. The experiment was conducted under greenhouse conditions ( $25 \pm 5.3/10 \pm 3.2$  °C day/night temperature, relative humidity of 60/80% during the light/dark periods, 16/8 h light/dark regime and an average photosynthetic photon flux density between 400 and 1200  $\mu\text{mol}/\text{m}^2/\text{s}$ ) at the Centre of Biotechnology of Borj Cedria (CBBC), Tunisia. To keep the soil moist, experimental pots were



**Fig. 1.** Geographic distribution of the studied accessions

irrigated every 2 days with distilled water until germination. Seedlings were watered as needed and fertilized weekly with a Hewitt nutrient solution (Hewitt, 1966) throughout the rest of study. After 30 days, fresh leaves of each individual were collected separately for DNA extraction.

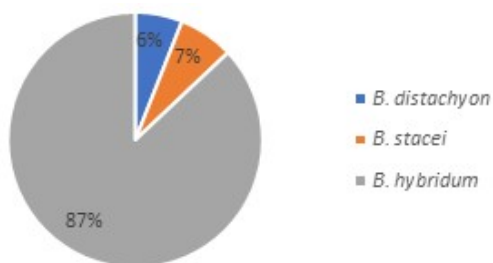
### 2.2 Molecular analysis

The DNA of the 360 accessions was extracted from about 20 mg of fresh leaves according to Geuna et al, (2003). A simple PCR amplification by the powerful SSR marker (ALB 165) was used

**Table 1:** Mean values of the 19 bioclimatic and longitude, latitude and altitudinal variables analysed for environmental variation of *Brachypodium* complex (*B. distachyon*, *B. stacei*, and *B. hybridum*)

Environmental variable	Code	Environmental variable	Code
Annual mean temperature	Bio1	Annual precipitation	Bio12
Mean diurnal range	Bio2	Precipitation of wettest month	Bio13
Isothermality	Bio3	Precipitation of driest month	Bio14
Temperature seasonality	Bio4	Precipitation seasonality	Bio15
Maximum temperature of warmest	Bio5	Precipitation of wettest quarter	Bio16
Min temperature of coldest month	Bio6	Precipitation of driest quarter	Bio17
Temperature annual range	Bio7	Precipitation of warmest quarter	Bio18
Mean temperature of wettest quarter	Bio8	Precipitation of coldest quarter	Bio19
Mean temperature of driest quarter	Bio9	Longitude	Long
Mean temperature of warmest	Bio10	Latitude	Lat
Mean temperature of coldest quarter	Bio11	Altitude	Alt

to determine the affiliation of each accession to the three species of the *Brachypodium* complex (*B. distachyon*, *B. stacei* and *B. hybridum*). The amplification products were analyzed in 2.5% agarose gel. According to Giraldo et al, (2012), this simple PCR-based alternative approach is reliable for an unequivocal distinction between the complex of species.



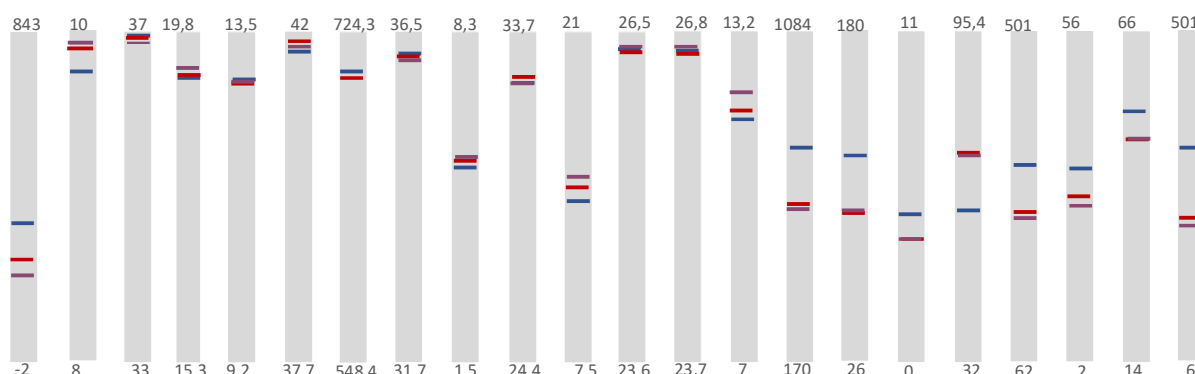
**Fig. 2.** Proportion of the three species in Tunisia

### 2.3 Environmental variation

Current climatic data from each occurrence point were extracted using the program DIVA-GIS v7.5. Comparison analyses from current data aimed to detect significant environmental differences among the three species for the 19 bioclimatic and three geographical (altitude, latitude, longitude) variables studied. To discriminate the three species in the environmental space and to identify the variables that most contributed to their differentiation, a principal component analysis (PCA) of extracted data was performed using the program Excel Stat 2021.

## 3. RESULTS

### 3.1 Identification and geographic distribution of *B. distachyon* complex in Tunisia



**Fig. 3.** Environmental variation of the *Brachypodium distachyon* complex species (*B. distachyon*, blue; *B. stacei*, red; *B. hybridum*, purple lines). Vertical bars represent the altitude, longitude, latitude and the 19 climatic variables studied (bio1 to bio19) respectively, with the species' mean values indicated by solid lines. Numbers indicate the minimum and maximum values for each environmental variable.

Based on the results of analysis with ALB 165, the allotetraploid species *B. hybridum* is widespread species in Tunisia. The two diploid species *B. distachyon* and *B. stacei* were found to be quite rare. Of the 360 analyzed accessions, 314 are allotetraploid (*B. hybridum*) whereas only 46 accessions are diploids (21 are *B. distachyon* and 25 are *B. stacei*) (Fig. 2.). Contrary to *B. distachyon* accessions which were found primarily in three populations (Ain Drahem, Kef, and Boukornine) and *B. stacei* accessions which were found in seven populations (Fayedh, Enfidha, Tabarka, Zaghouan, Ain Drahem, Takelsa and Boukornine), the allotetraploid species *B. hybridum* was characterized by a scattered distribution extending from the southern to the northern region of Tunisia. In some populations like Silyana, Amdoun, Kandar, Mahres and Djerba, accessions are exclusively *B. hybridum*. The sympatric occurrences between the three species were found only in Ain Drahem and Boukornine populations.

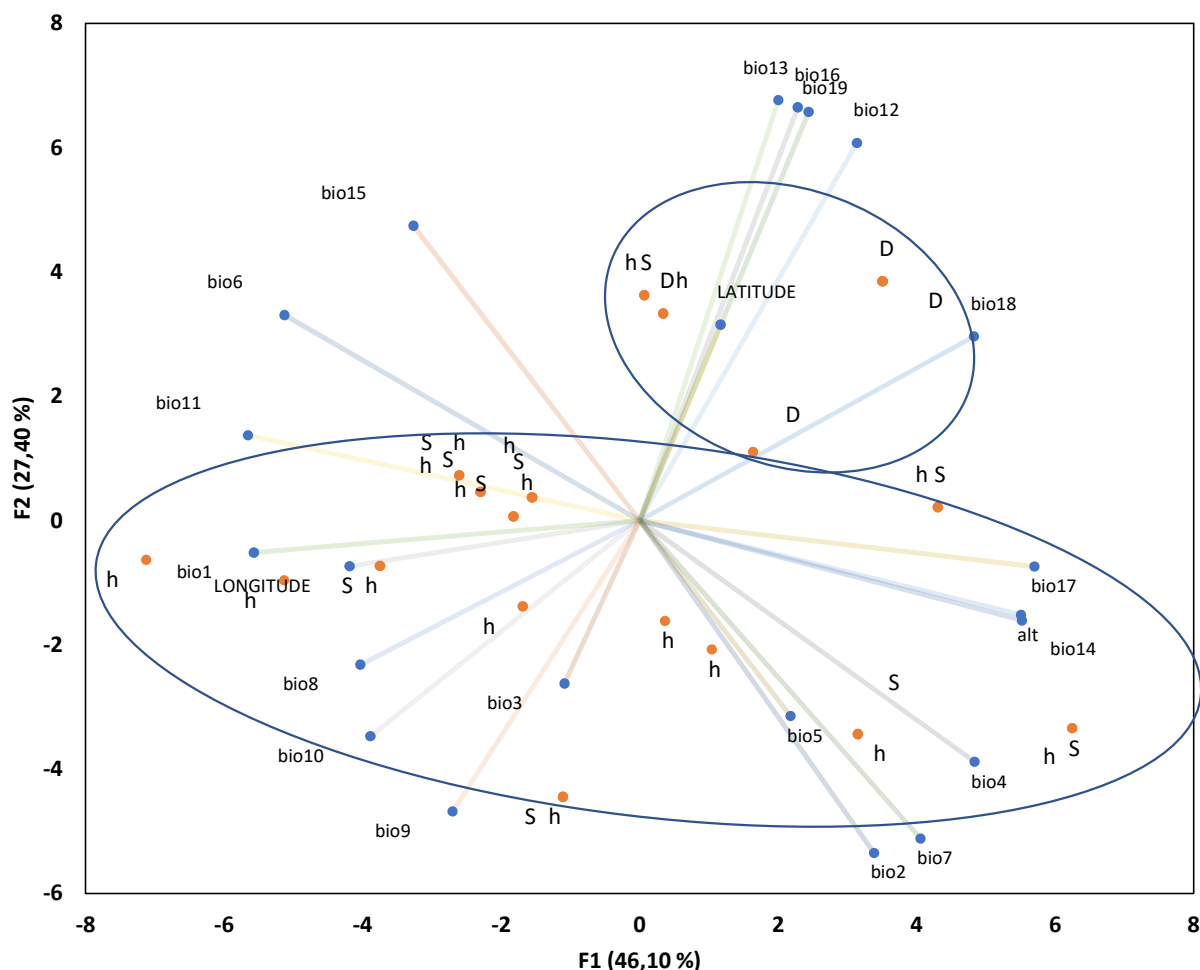
### 3.2 Environmental variation of *B. distachyon*, *B. stacei*, and *B. hybridum*

Despite the difference in the bioclimatic stage of the "*B. distachyon*" s.l. complex taxa, no fundamental environmental differentiation for its three species were found. Nonetheless, *B. stacei* showed intermediate values for the majority of variable and means for *B. distachyon* are higher or lower than the other two species (Fig. 3.).

The environmental space defined by the two first PCA axes showed a relative differentiation of the *B. distachyon* and *B. stacei* samples along the opposite extremes of the second axis (27.4% variance), whereas those of *B. hybridum* overlapped with both groups but more greatly

with the *B. stacei* cluster (Fig. 4.). The variables that most contributed to the first PCA axes were altitude, bio1, bio6, bio11, bio14, bio17 and bio18 (PCA1), bio12, bio13, bio16 and bio17 (PCA2) and bio3 (PCA3).

classified *B. stacei* (7%) and *B. distachyon* (6%). The latter is limited to the region of Ain Draham, Elkef and Djebel Boukarnin. The strong dominance of polyploids in almost all sites, including the driest sites, is consistent with the



**Fig. 4.** Bidimensional principal component analysis (PCA) plot of *B. distachyon* (D), *B. stacei* (S), and *B. hybridum* (h) records based on data from 19 bioclimatic variables and Longitude and Latitude. PCA 1 and PCA 2 accounted for 46.1% and 27.40% of the variance, respectively

#### 4. DISCUSSION

*Brachypodium* ssp is a small annual grass in which the existence of three different cytotypes (with 10, 20 or 30 chromosomes) has long been considered as a series of autopolyploidy with  $x = 5$ . However, it has been shown that the cytotypes which were presumed polyploids, actually represent two distinct species of the genus *Brachypodium*, recently named *Brachypodium stacei* ( $2n = 2x = 20$ ) and *Brachypodium hybridum* ( $2n = 4x = 30$ ) (Giraldo et al. 2012). In this study, we used the ALB 165 marker to distinguish between the three species. The results show that the majority of accessions in Tunisia are *Brachypodium hybridum* (87%), on the other hand, relatively few of them were

idea that polyploids take advantage of their genetic potential and can adapt to a wide range of environmental conditions (Comai, 2005; Te Beest et al., 2012; del Pozo and Ramirez-Parra, 2015). The first karyological studies carried out on material indicated the existence of  $2n = 10$ ,  $2n = 20$  and  $2n = 30$  cytotypes, and  $2n = 30$  being the most common (Robertson, 1981). According to Antonio et al., (2011) the distribution of diploids ( $2n = 10$ ) and allotetraploids ( $2n = 30$ ) in this species is geographically structured throughout its range in the Iberian Peninsula and is associated with gradients of aridity. After controlling the geographic and altitudinal effects, the link between aridity and polyploidization persisted in own collection. In addition, the

amount of significant environmental differences found between *B. distachyon* and *B. stacei* (Fig. 3. and Fig. 4.) suggest distinct adaptations to different ecological tolerances in these diploids' specie.

Environmental niche analysis indicated that, overall, *B. distachyon* grows in higher, cooler, and wetter places north of 36°; *B. stacei* in lower, warmer, and drier places; and *B. hybridum* in places with intermediate ecological features and across latitudinal boundaries but also overlapping with those of its parents, more often with those of *B. stacei*. Such results were corroborated with those found by López-Alvarez et al., 2015 and Catalán et al., 2016. This concurs with the finding that most *B. distachyon* lines require vernalization treatment in order to flower, whereas *B. stacei* and *B. hybridum* lines do not (Vogel et al., 2009). This finding of geographical segregation between diploids and auto- and allopolyploids has been repeatedly described in many species (e.g. Husband and Schemske, 1998; Baack, 2004; see also Levin, 2002), including grasses (Keeler, 1990; see also Gibson, 2009). Thus, the wide dispersion of *B. hybridum* could be due to the benefit of multiple genomes resulting from polyploidization (Beest et al., 2012). The narrow range observed for *B. stacei* and *B. distachyon* suggests specific habitat requirements for these species and makes them less likely that their restricted distribution results mainly from limited migration / colonization processes.

## REFERENCES

- Baack E.J. (2004). Cytotype segregation on regional and microgeographic scales in snow buttercups (*Ranunculus adoneus*: Ranunculaceae). *American Journal of Botany* 91, 1783-1788.
- Catalán P., López-Alvarez D., Bellosta C., Villar L. (2016) - Updated taxonomic description, iconography and habitat preferences of *Brachypodium distachyon*, *B. stacei* and *B. hybridum* (Poacea). *Anales Del Jardin Botanico De Madrid* 73, e028.
- Catalán P., Muller J., Hasterok R., Jenkins G., Mur L.A.J., et al. (2012) - Evolution and taxonomic split of the model grass *Brachypodium distachyon*. *Annal of Botany*. 109, 385-405.
- Comai L. (2005) - The advantages and disadvantages of being polyploid. *Nature Reviews* 6, 836-846.
- Del Pozo J.C. and Ramirez-Parra E. (2015). Whole genome duplications in plants: An overview from *Arabidopsis*. *Journal of Experimental Botany*, 66, 6991-7003.
- Garvin D.F., Gu Y.Q., Hasterok R., Hazen S.P., Jenkins G., Mockler T.C., Mur L.A.J., and Vogel J.P. (2008) - Development of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for grass crop research. *Crop Science* 48, 69 - 84.
- Garvin D.F., Gu Y.Q., Hasterok R., Hazen S.P., Jenkins G., Mockler T.C., et al. (2008) - Development of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for grass crop research. *Crop Science*. 2008; 48: 69-84
- Geuna F., Toschi M. and Bassi D. (2003) - The use of AFLP markers for cultivar identification in apricot. *Plant Breed.* 122, 526-531.
- Gibson D.J. (2009) - Grasses and grassland ecology. New York, NY, USA: Oxford University Press.
- Giraldo P., Rodríguez-Quijano M., Vázquez J.F., Carrillo J.M. and Benavente E. (2012) - Validation of microsatellite markers for cytotype discrimination in the model grass *Brachypodium distachyon*. *Genome* 55, 523 - 527.
- Hasterok R., Draper J. and Jenkins G. (2004) - Laying the cytotoxic foundations of a new model grass, *Brachypodium distachyon* (L.) beauv. *Chromosome Res.* 12, 397-403.
- Hewitt E.J. (1966) - Sand and water culture methods used in the study of plant nutrition. *Tech. Commun.* 22. Rev. 2nd ed. Commonw. Bur. Hort. Plant. Crops, East Malling, UK.
- Husband B.C. and Schemske D.W. (1998) - Cytotype distribution at a diploid-tetraploid contact zone in *Chamerion (Epilobium) angustifolium* (Onagraceae). *American Journal of Botany* 85, 1688-1694.
- Idziak D., Hazuka I., Poliwczak B., Wiszynska A., Wolny E., et al., (2014) - Insight into the karyotype evolution of *Brachypodium* species using comparative chromosome barcoding. *PLoS One* 9: e93503.
- Keeler K.H. (1990) - Distribution of polyploid variation in big bluestem (*Andropogon gerardii*, Poaceae) across the tallgrass prairie region. *Genome* 33, 95 - 100.
- Levy A.A. and Feldman M. (2002) - The impact of polyploidy on grass genome evolution. *Plant Physiology* 130, 1587- 1593.
- López-Alvarez D., Manzaneda A.J., Rey P.J., et al. (2015) - Environmental niche variation and evolutionary diversification of the *Brachypodium distachyon* grass complex

- species in their native circum Mediterranean range. *American Journal of Botany* 102, 1–16.
- Manzaneda A.J., Rey P.J., Bastida J.M., Weiss-Lehman C., Raskin E. and Mitchell-Olds T. (2012) - Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytologist* 193, 797 – 805.
- Manzaneda A.J., Rey P.J., Bastida J.M., Weiss-Lehman C., Raskin E. and Mitchell-Olds T. (2011) - Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New phytologist*. 193, 797-805.
- Robertson I.H. (1981) - Chromosome numbers in *Brachypodium* Beauv. (Gramineae). *Genetica* 56, 55-60.
- Schippmann U. (1991) - Revision der europäischen Arten der Gattung *Brachypodium* Palisot de Beauvois (Poaceae), vol. 45. Conservatoire et Jardin Botaniques de Genève, Geneva, Switzerland.
- Te Beest M., Le Roux J.J., Richardson D.M., Brysting A.K., Suda J., Kubesova M. and Pysek P. (2012) - The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109, 19 – 45
- Tyler L., Lee S.J., Young N.D., Delulio G.A., Benavente E. et al., (2016) - Population structure in the model grass is highly correlated with flowering differences across broad geographic areas. *Plant Genome* 9: doi: 10.3835/plantgenome2015.08.0074.
- Vogel J.P., Tuna M., Budak H., Huo N.X., Gu Y.Q., Steinwand M.A. (2009) - Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. *BMC Plant Biology* 9, 88.