Plant Physiology and Biochemistry 143 (2019) 246–256

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Genetic variability of morpho-physiological response to phosphorus deficiency in Tunisian populations of *Brachypodium hybridum*

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ARTICLE INFO

Keywords:
Brachypodium hybridum
Genetic variability
Phosphorus deficiency
Adaptive differential response
Direct selection

ABSTRACT

Brachypodium hybridum (*Poaceae*) is widely distributed in the dry environments in Mediterranean basin, due to its high tolerance to drought. Investigating the natural variation of *B. hybridum* in response to environmental stresses is crucial for unraveling the genetic network of its stress tolerance. 79 *B. hybridum* lines from eight Tunisian populations were screened for their performance to low P availability using morpho-physiological parameters. ANOVA showed that treatment and population*treatment factors were the most contributors in the explained variance for the majority of parameters. A considerable population differentiation was detected in control and under P level (Qst = 0.77 vs Qst = 0.62). This suggests that *B. hybridum* exhibit an adaptive differential response to P deficiency related environmental conditions. Results revealed that Raouad and Sejnen lines were the most tolerant to P deficiency followed by Haouaria and Enfidha lines. The remaining populations were classified as sensitive. This pattern suggests that coastal populations were more tolerant to P deficiency than the inland ones. A slightly higher heritability was evidenced under low P level for most of traits, indicating that the direct selection under P deficiency is more reliable than an indirect one under optimal P supply.

1. Introduction

Phosphorus (P) is vital for all living organisms as it is represents a structural component of nucleic acids and involved in many energy-related processes (Pang et al., 2018). For plants, P plays a crucial role in diverse physiological and metabolic processes such as photosynthesis, carbohydrate metabolism, respiration, glycolysis, and membrane synthesis and integrity and many other biochemical reactions (Sulieman and Tran, 2015). Hence, limiting P conditions may gravely affect plant growth and development. With the ongoing climate change, the growing world population and the urgent need to increase the food production, improving P efficiency has become a worldwide challenge. Screening for efficient varieties, cultivars or genotypes tolerant to P limiting conditions could be a cost-effective and attractive strategy for ensuring a long-term sustainability of agricultural systems.

The two diploid species *B. distachyon* (x = 5, 2n = 10) and *B. stacei* (x = 10, 2n = 20) and their derived allotetraploid *B. hybridum* (x = 5 + 10, 2n = 30) are a complex of species belonging to the Poaceae family, characterized by their close relationship to the major cereals critical for global food security such as wheat, maize, rice, barley. These three annual species present many common features that allow them to be a promising model system for physiological and functional genomics of temperate cereals and forage grasses, including small genome size, short life-cycle, small stature and easy handling in controlled conditions. Since the accomplishment of genome sequencing of *B. distachyon* and the deciphering of the taxonomical relationship between the tree species (López-Alvarez et al., 2012), many worldwide natural populations have been collected and several studies have been conducted in order to unravel the patterns of their geographical distribution (Lopez-Alvarez et al., 2015) and genetic diversity (Neji et al., 2015; Vogel et al., 2009) as well as their physiological and molecular responses to various environmental stresses such as Drought (Verelst et al., 2013), salinity (Kim et al., 2012) and nutrient deficiency (Poiré et al., 2014). Investigations have demonstrated that the allotetraploid species *B. hybridum* is more robust and displays greater adaptation to harsh environmental constraints, which may explain its wider

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https://doi.org/10.1016/j.plaphy.2019.09.006
Received 2 July 2019; Received in revised form 3 September 2019; Accepted 4 September 2019
Available online 05 September 2019
0981-9428/

geographical distribution compared to it diploid progenitors (Manzaneda et al., 2012). To our knowledge, the natural variation of the *B. distachyon* and its closely related species to nutrient deficiency remains poorly understood. Recently, a promising research project focusing on the natural phenotypic variability in nitrogen (N) and phosphorus (P) response in diverse *B. distachyon* accessions has been initiated in order to provide the baseline of further investigations towards unraveling the genetic basis of the response to nutrient deficiency in the grasses (Poiré et al., 2014). Based on yield-related traits (biomass production and shoot and roots growth) and physiological traits (photosynthetic capacity and N and P concentration in leaves), the first results of this work have revealed a considerable genetic variation in N and P use efficiency in *B. distachyon* (Poiré et al., 2014). The same authors have demonstrated that the shoots and roots growth are strongly influenced by P availability and the photochemical efficiency is considerably compromised under P starvation (Poiré et al., 2014). More recently, Zhao et al. (2018) have demonstrated that short term P-limited conditions (10 μM for 7 days), P content decreased significantly in roots but not shoots. However, the roots and shoots growth was not significantly affected. Taken together, these findings suggest that screening *Brachypodium* lines for their phosphorus efficiency should be performed after a long-term P treatment and must involve diverse morpho-physiological parameters.

Given the wide geographical distribution of the allotetraploid species *B. hybridum* in its natural geographical range and its greater adaptability to harsh environmental conditions compared to its two diploid progenitors species, we hypothesize that the natural populations of this species display an extensive genetic variation in response to P deficiency. In the present study, diverse *B. hybridum* lines collected from diverse Tunisian populations were screened for their response to P deficiency using a large set of morpho-physiological parameters in order to identify lines and/or populations and parameters that could be exploited in establishing future breeding programs and exploring the genetic basis of the response to P deficiency in grasses.

Fig. 1. Geographical locations of Tunisian populations of *Brachypodium hybridum*.

2. Material and methods

2.1. Plant material and growth conditions

The plant material used in this study consists of seventy nine lines of *Brachypodium hybridum* originated from eight distinct locations in Tunisia; Douar El Haj Wniss, Fayedh, Enfidha, Hawaria, El Kef, Raouad, Sejnen and Jbal Zaghouan (Table 1 and Fig. 1). These lines were selected from a total of 145 lines collected from nine populations morphologically and genetically characterized using a large set of morpho-phenological features and simple sequences repeats (SSR) markers (Neji et al., 2015a, 2015b). The population of Ain Drahem was discarded from the present study due the few lines available (Neji et al., 2015b). Except for the population of Fayedh, in which only 9 lines were available, 10 lines were selected from each of the remaining populations for the present study. The seeds of all the lines used in this study were obtained after two generations of a spontaneous selfing under controlled conditions. The selection of lines analyzed for this study was based on the maximization of the morphological and diversity within each population. Each of the selected lines was cultivated in a plastic pot filled with 2.5 kg of a sterilized sandy-loam soil with a pH of 6.9 for two months under glasshouse conditions at the Faculty of Sciences of Gabès, Tunisia with two phosphorus (P) treatments: 1 mM (control) and 5 μM (P deficiency). The temperature of the glasshouse was set at 25 °C/20 °C (day/night), the relative humidity was at 65% and the photoperiod was set at 16 h/8 h light/dark cycle. At the beginning, four to five seeds from each line were sown and irrigated with distilled water for one week. Seedlings were then thinned to three morphologically homogenous replicates in both, control and under P deficiency conditions, and irrigated with 1/10-strength Hoagland's nutritive solution containing 1 mM KH_2PO_4, 2.5 mM Ca $(NO_3)_2$, 2.5 mM KNO_3, 1 mM $MgSO_4$, 10 μM Fe (as FeEDTA), 23 μM H_3BO_3, 0.16 μM $CuSO_4.5H_2O$, 0.38 μM $ZnSO_4.7H_2O$, 4.6 μM $MnCl_2.4H_2O$, and 0.06 μM $H_2MoO_4.1H_2O$

Table 1
Geographical coordinates of the Tunisian populations of *Brachypodium hybridum* and their climatic regions.

ID	Location	Latitude	Longitude	Distance to coastline (Km)	Bioclimatic stage
F	Fayedh	35°4′4.1″N	9°40′32.1″E	116.3	Arid
E	Enfidha	36°7′8.04″N	10°27′44.90″E	0.55	Lower semi-arid
Z	Jbal zaghouan	36°22′6.80″N	10° 5′20.00″E	39.1	Upper semi-arid
H	Haouaria	37° 2′32.54″N	10°59′38.58″E	2	Sub-humid
R	Raoud	36°57′0.00″N	10°14′14.00″E	0.2	Upper semi-arid
S	Sejnen	37°4′38.90″N	9° 9′51.10″E	9.8	Humid
K	El Kef	36° 6′36.80″N	8°37′39.00″E	165.7	Lower semi-arid
D	Douar El Haj Wniss	35°40′56.40″N	8°53′49.00″E	193.2	Upper semi-arid

for two weeks. This solution was used to continuously irrigate the control plants until the end of the experiment, while the treated plants were irrigated with a Hoagland's solution containing only 5 µM KH_2PO_4. For these latter, the potassium (K) concentration was maintained by the addition of KCl. A completely randomized design with 2-factorial arrangement of populations and two phosphorus levels was employed in our experiment.

2.2. Morpho-physiological characterization under greenhouse conditions

At the end of experiment, all the cultivated lines were screened for a set of 12 traits related to the plant morphology, 4 traits related to gas exchange (photosynthetic parameters) and 6 related traits to Phosphorus efficiency (PE traits). The morphological variables included shoots fresh weight (SFW), roots fresh weight (RFW), shoots dry weight (SDW), roots dry weight (RDW), total dry matter (TDM), shoot by root dry weight (SDW/RDW), average length of tillers (ALT), root length (RL), number of tillers (NL), number of leaves (NL), average of leaf length (ALL), total number of spikelets (TNS). The photosynthetic parameters, including the stomatal conductance (*gs*), the intercellular CO_2 concentration (Ci) and the net photosynthetic rate (Pn), were measured in healthy leaves between 10:00 and 17:00 in a fully sunny day using a portable computerized open gas system (LI-6400, LiCor, Lincoln, NE, USA). These leaves were then harvested, conserved at −80 °C and used later to quantify the Acid Phosphatase Activity (APA) according to Kouas et al. (2009). Moreover, the P concentrations in roots and shoots were colorimetrically measured in 60 mg of dry vegetal material digested using a mixture of $HNO_3/HClO_4$ (4:1) following the vanadomolybdate method (Fleury and Leclerc, 1943). The latter were multiplied by the dry weights relative to each organ to determine the root and shoot P contents (RPC and SPC, respectively). The measurements were performed in 3 replicates for the morphological parameters, whereas only 2 replicates used for those related to gas exchange and phosphorus status of plants.

2.3. Phosphorus efficiency parameters

To investigate the performance of the studied lines in terms of efficiency and responsiveness, we calculated the four following parameters: P acquisition efficiency (PAE), P utilization efficiency (PUE), relative efficiency of phosphorus use (REP) and P stress factor (PSF). The PAE, which refers to the ability of plant to accumulate P in its shoots, was calculated as the P content in the whole plant (PPC) divided by the roots dry weight (Wang et al., 2010), whereas PUE which, according to (Pan et al., 2008), refers to the capacity of plant to produce biomass using the acquired P, was calculated as the ratio between the whole plant dry weight and P content in the whole plant. The REP for each line was calculated as the ratio between the whole plant dry weights under P deficiency and control conditions ($\text{REP}\ (\%) = \frac{\text{PDW at 5}\ \mu\text{M P}}{\text{PDW at 1 mM P}} * 100$) (Ozturk et al., 2005). Finally, we calculated the P-stress factor (PSF), which refers to the relative reduction in biomass due to P deficiency (Akhtar et al., 2008), using the following formula: $\text{PSF}\ (\%) = \frac{\text{TDM at 1 mM P} - \text{TDM at 5}\ \mu\text{M P}}{\text{TDW at 1 mM P}} * 100$.

2.4. Data analysis

First, the data of all the parameters were subjected to a descriptive statistical analysis in order to characterize the patterns of variation within and among populations in control and under P deficiency conditions. We then conducted oneway and two-way ANOVAs using type III sums of squares to investigate the effects of the population, line, treatment and their interactions on all the analyzed traits. Pearson correlation analysis (*r*) was performed to analyze the relationship among all parameters pairwise in both studied conditions. Tukey's *t*-test was used for pairwise comparisons was and $P < 0.05$ was set as significance threshold. All these analyses were computed using XLSTAT version 2018.1. Moreover, we employed the ‘varcomp’ function in the ‘ape’ R package (Paradis et al., 2004) using restricted maximum likelihood to retrieve the proportion of the total variance explained by within (Vwp) and among populations (Vap) components and the residual variance (V_{error}) component, which refers to the environmental effect. for all analyzed traits. The variance components were then used to calculate between-population variation (Qst) as Qst = Vap/(Vap + Vwp) and the broad sense heritability (H^2) as H^2 = Vwp/(Vwp + V_{error}) according to Neji et al. (2015a).

Furthermore, we performed the two multivariate analyses principal component analysis (PCA) and hierarchical clustering analysis (HCA) in order to examine the grouping of the analyzed traits and the patterns of differentiation between lines and populations in control and under P deficiency conditions. The PCA was conducted using XLSTAT version 2018.1 on the basis of the standardized mean values of all analyzed traits. The ordination of the studied lines was visualized using a bi-dimensional plot formed by the first two principal components (PC1 and PC2). The HCA was conducted using the “gplots” and “heatmap.2” functions in R software version 3.4.2 (https://www.r-project.org/). In this analysis, the function “dist” was first used to calculate Manhattan distances among all studied lines and then the hierarchical cluster analysis was performed using “hclust” function. By default, the complete linkage method was used as clustering method.

Finally, in order to classify the studied lines in terms of efficiency and responsiveness to low P availability, the REP, PAE, PUE and PSF were plotted against the total dry matter (TDM) at 5 µM P. The generated ordination plots were then used to separate the lines into four categories, as described previously (Neto et al., 2016): (i) efficient and non-responsive (ENR); (ii) efficient and responsive (ER); (iii) non-efficient and responsive (NER); and (iv) non-efficient and non-responsive (NENR). The lines presenting a total dry matter higher than the average of all studied lines were considered as efficient and the lines displaying REP, PAE, PUE and PSF values higher than the average were categorized as responsive and vice versa.

3. Results

3.1. Morpho-physiological variation

To assess the morpho-physiological variation among and within populations, we considered the coefficient of variations (CV) of all analyzed traits separately in control and P deficiency conditions. Overall, our results revealed a considerable genetic variation for most of analyzed traits in both treatments, with a greater level of variation detected among populations rather than within populations (Supplementary file 1). Among populations, all analyzed traits showed moderate to high CV, ranging from 4.42% to 37.94% in control conditions and from 5.53% to 65.03% under P deficiency. Means CV of all analyzed parameters were 15.53% and 28.21% in both conditions respectively. Noticeably, CVs of all traits were considerably much higher under P deficiency than in control conditions, indicating that the morpho-physiological variation of Tunisian *Brachypodium hybridum* populations was strongly affected by P deficiency. In control conditions, the highest levels of variation were observed in ALL, NL, TNS and NT with CVs ranging from 23.97% to 37.94%, respectively, whereas the lowest level of variation was observed for the intercellular CO_2 concentration (CV = 4.22%). Under P deficiency, the CVs of most traits exceeded 20%. The highest levels of variation were observed for SDW/RDW, SPC/RPC, SDW and ALL with CVs of 65.03%, 54.67%, 50.96% and 40.86%, respectively. Remarkably, in both control and P deficiency conditions, the morphological traits were found the most variable (18.59% and 30.98%, respectively) when compared to PE traits (13.83% and 28.02%, respectively) and the photosynthetic parameters (9.36% and 20.24% respectively) (Supplementary file 1). Within populations, a relatively low and comparable amount of variation was observed in all the studied populations for most of analyzed traits, with mean CVs in control conditions ranging from 5.28% in Raouad to 9.82% in Douar El Haj Wniss. A similar pattern was also observed under P deficiency, with Jbal Zaghouan and Raouad presenting the highest and the lowest average CVs (10.80% and 6.94%, respectively) (Supplementary file 1). Meanwhile, means comparison showed that the populations differed significantly for all analyzed traits in both control in P limiting conditions ($p \leqslant 0.05$). In control conditions, results revealed that Raouad and Sejnen populations displayed the strongest vegetative growth as they produced the longest tillers and the highest number of leaves and tillers, and exhibited relatively high root development in terms of weight and length. These two populations were also characterized by the high levels of SPC, RPC and TPC, suggesting that the phosphorus availability enhanced the vegetative growth of these populations. Compared to the two aforementioned populations, those originating from Douar El Haj Wniss, El Kef, Fayedh and Haouaria were characterized by less important P accumulation in their shoots and roots but produced higher dry biomass, indicating that these populations displayed thicker tillers and leaves. Moreover, Sejnen population was characterized by the most important underground growth as it exhibited the highest RDW and RL, followed by the populations Enfidha, Raouad and El Kef. Noteworthy, Haouria population displayed a smallest under and aboveground development, indicating that its high dry biomass was mainly related to its high seed production. On the other hand, our results showed the studied populations differed significantly on the magnitude of their morpho-physiological response to P limiting conditions. Generally, except for RL and APA, all parameters were affected in all the studied populations. Remarkably, the populations originated from Haouria, Enfidha, Raouad and Sejnen showed only a small reduction in most of analyzed traits. This pattern indicates these latter were able to conserve a relatively high aboveground growth due their substantial shoots P allocation, considerable gas exchange and increased Acid Phosphatase Activity under P deficiency. In contrast, Douar El Haj Wniss Fayedh, El Kef, and Jbal Zaghouan populations were especially characterized by a small reduction in their RDW, an increase in their root length and very similar P allocation in shoots and roots, resulting in a considerable root development and a relatively low biomass production under P deficiency.

Furthermore, ANOVA revealed a significant individual effect of line, population and treatment in the total variation explained for all the analyzed parameters ($P < 0.05$), with one exception in the population effect (*gs*) and two exceptions in the treatment effect (*gs* and NT). A significant contribution of population*treatment and line*treatment interactions to the total variation was also observed for each of analyzed parameters, except for the stomatal conductance (*gs*) (Table 2). The extent to which each of the four factors contributed in the variation of the analyzed parameters varied considerably, ranging from 0.43% to 68.9% for population, from 0.42% to 92.9% for treatment, from to 0.16%–20.43% for line, from 0.29% to 54.19% for population-treatment interaction, and from 0.11% to 7.89% for treatment-line interaction. The highest amounts of variation explained by the population effect were found for LL, NL and RFW (>50%). The treatment strongly influenced some morphological parameters related to biomass production (SFW, SDW, PDW, ALT and NT) and some parameters related to P efficiency such as SPC, RPC, PPC SPC/RPC and APA (Fig. 2). Taken all the parameters together, results showed that, the main source of variation was the treatment factor (44.09%) followed by the population and population*treatment factors (28.17%, 18.33%, respectively). Noticeably, line and line*treatment effects were generally less important and contributed almost equally with a very low amount in the total explained variation (4.39% and 3.34%, respectively).

3.2. Heritability and correlations between parameters

Overall, the broad-sense heritability (H^2) was found relatively high for most analyzed traits in both control and under P deficiency. It ranged from 0.17 to 0.94 and 0.12 to 0.99, in both conditions, respectively (Table 2). In control conditions, the highest levels of heritability were observed in the morphological traits, followed by PE traits and the photosynthetic parameters, with values averaging for each set of parameters in 0.78, 0.64 and 0.27, respectively. This pattern indicates that the variation of first two sets was mainly governed by genetic factors under control conditions. The low-phosphorus treatment significantly increased the heritability of all analyzed traits, except for SFW and Fv/Fm. In addition, we noticed that, under P deficiency, all the morphological traits and those related P efficiency displayed comparable levels of heritability, with average values of 0.99 and 0.94, respectively. However, the average heritability value for the photosynthetic parameters was found slightly higher than that observed in control conditions (0.3 vs 0.27), which indicates that, in control and under deficiency conditions, the variation of these parameters was mostly influenced by environment and only a small part was associated to genetic factors. Overall, the average heritability value for all analyzed parameters was much higher under P deficiency conditions than in control conditions (0.73 vs 0.41), suggesting that the response to P deficiency in the Tunisian *B. hybridum* lines was mainly controlled at the genetic level.

Moreover, correlation between pairwise of all the parameters measured in control and under P deficiency was analyzed using Pearson's coefficients. In control conditions, among the 253 possible correlations, a total of 129 significant correlations were recorded, 78 of them were positive. Noticeably, the highest positive correlations were recorded between PE parameters pairwise such as SPC and PPC ($r = 0.98$; <0.0001), SPC and RPC ($r = 0.86$; <0.0001) and RPC and PPC ($r = 0.62$; <0.0001). Relatively high positive correlations were also observed between pairwise of morphological traits related to plant vigor and biomass production and parameters related phosphorus accumulation such as NT and ALL ($r = 0.82$; <0.0001), PW and SDW ($r = 0.82$; <0.0001), ALT and ALL ($r = 0.81$; <0.0001), PPC and ALT (0.76; <0.0001) and NL and PPC ($r = 0.70$; <0.0001). On the other hand, among the 51 significant negative correlations, the highest ones were observed between PUE and all the traits related to P status of plant (SPC, RPC and PPC) and some morphological traits (ALT, ALL, NT and RL). Significant negative correlations were also recorded the between photosynthetic parameters and most of morphological traits (Table 3). These results suggest that, in control conditions, the phosphorus availability in the different organs largely improved the plant growth and biomass production more than the photosynthetic parameters. Under P deficiency, all traits related to belowground biomass allocation, those related to photosynthetic process and the PE related traits were found significantly positively correlated to each other, indicating that these traits were mutually affected by the P deficiency. However, while displaying strong positive correlations between each other, roots related traits (RL, RDW and RPC) were showed strong negative relationship with all remaining traits. This result pointed towards an increase of root length and biomass under P deficiency and suggested that the ability of seedlings to absorb P from soil was closely related to root morphology.

3.3. Population differentiation and structure of the morpho-physiological variation in control and under P deficiency conditions

For all analyzed parameters, results showed a moderate to high differentiation among the eight studied populations in the two studied conditions with Qst values ranging from 0.23 (SDW/RDW) to 0.99 (SPC) in control conditions and from 0.24 (*gs*) to 0.95 (LL) under P deficiency (Table 2). Overall, the studied populations were found more distinct from each other under P deficiency than in control conditions (average Qst of 0.77 and 0.62, respectively). Noticeably, 17 among the 22 analyzed parameters displayed higher Qst values under P deficiency, in comparison with the control conditions, indicating a considerable difference between the studied populations in response to P deficiency.

Table 2
ANOVA, heritability (H^2) and coefficient of among populations differentiation (Qst) for all analyzed traits in *Brachypodium hybridum* populations.

	Population		Treatment		population*Treatment		Line		Treatment*Line		H^2		Qst	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	1 mM	5 µM	1 mM	5 µM
SFW	22.13	< 0.0001	166.27	< 0.0001	25.29	< 0.0001	5.30	< 0.0001	5.95	< 0.0001	0.94	0.75	0.75	0.91
SDW	377.61	< 0.0001	298.87	< 0.0001	306.68	< 0.0001	277.98	< 0.0001	63.11	< 0.0001	0.64	1.00	0.83	0.82
RFW	97.79	< 0.0001	5.58	0.02	56.23	< 0.0001	28.01	< 0.0001	17.91	< 0.0001	0.69	0.99	0.69	0.44
RDW	83.37	< 0.0001	178.02	< 0.0001	126.07	< 0.0001	16.91	< 0.0001	26.86	< 0.0001	0.55	0.96	0.50	0.67
TDM	88.04	< 0.0001	6655.77	< 0.0001	50.15	< 0.0001	15.68	< 0.0001	10.93	< 0.0001	0.92	0.97	0.98	0.58
SDW/RDW	234.90	< 0.0001	106.28	< 0.0001	260.37	< 0.0001	49.16	< 0.0001	53.50	< 0.0001	0.69	0.96	0.23	0.84
ALT	162.91	< 0.0001	282.86	< 0.0001	40.93	< 0.0001	56.92	< 0.0001	18.60	< 0.0001	0.88	0.96	0.65	0.84
RL	179.24	< 0.0001	294.71	< 0.0001	122.52	< 0.0001	49.61	< 0.0001	34.48	< 0.0001	0.88	0.95	0.58	0.92
NT	171.03	< 0.0001	275.38	< 0.0001	23.02	< 0.0001	26.12	< 0.0001	4.74	< 0.0001	0.77	0.94	0.96	0.93
NL	120.64	< 0.0001	0.81	0.37	47.21	< 0.0001	37.41	< 0.0001	17.25	< 0.0001	0.90	0.92	0.64	0.83
ALL	259.85	< 0.0001	9.63	< 0.0001	66.98	< 0.0001	46.67	< 0.0001	13.32	< 0.0001	0.78	0.98	0.65	0.96
TNS	125.21	< 0.0001	42.18	< 0.0001	61.11	< 0.0001	28.27	< 0.0001	14.70	< 0.0001	0.80	0.94	0.82	0.94
SPC	150.26	< 0.0001	2148.86	< 0.0001	167.25	< 0.0001	47.24	< 0.0001	52.41	< 0.0001	0.66	0.97	0.99	0.77
RPC	64.19	< 0.0001	2899.13	< 0.0001	63.79	< 0.0001	17.32	< 0.0001	17.25	< 0.0001	0.60	0.96	0.88	0.72
TPC	45.77	< 0.0001	10503.04	< 0.0001	30.90	< 0.0001	10.65	< 0.0001	7.39	< 0.0001	0.86	0.93	0.28	0.45
SPC/RPC	117.85	< 0.0001	5844.83	< 0.0001	158.79	< 0.0001	20.86	< 0.0001	26.72	< 0.0001	0.68	0.99	0.28	0.92
APA	272.72	< 0.0001	1528.50	< 0.0001	259.54	< 0.0001	195.35	< 0.0001	189.40	< 0.0001	0.84	0.95	0.54	0.93
PUE	108.52	< 0.0001	116.557	< 0.0001	114.63	< 0.0001	27.17	< 0.0001	28.45	< 0.0001	0.48	0.93	0.80	0.83
PAE	111.12	< 0.0001	75.104	< 0.0001	91.30	< 0.0001	24.63	< 0.0001	21.40	< 0.0001	0.59	0.92	0.42	0.93
Fv/Fm	39.76	< 0.0001	35.38	< 0.0001	25.11	< 0.0001	7.13	< 0.0001	4.75	< 0.0001	0.27	0.12	0.59	0.87
Gs	1.87	0.07	2.56	0.11	1.84	0.08	4.18	< 0.0001	4.11	< 0.0001	0.33	0.38	0.35	0.25
Pn	61.52	< 0.0001	93.67	< 0.0001	50.90	< 0.0001	14.68	< 0.0001	12.40	< 0.0001	0.33	0.37	0.26	0.64
Ci	15.91	< 0.0001	0.27	0.60	28.58	< 0.0001	5.13	< 0.0001	5.61	< 0.0001	0.17	0.33	0.71	0.76

The most important increases in Qst values were recorded for SDW/RDW (0.23–0.83), SPC/RPC (0.28–0.92), PAE (0.41–0.93), Pn (0.25–0.63) and APA (0.53–0.92).

The dataset derived from all morpho-physiological traits were analyzed by principal component analysis and hierarchical clustering analysis in order to unravel key contributors to the variation among the studied populations under the two phosphorus levels. Under control conditions, PCA showed that the variation among populations was significantly explained by the first two axes (PC1 and PC2; Eigenvalues of 6.34 and 3.84, respectively), representing 37.11% and 12.67%, of the variance, respectively. Except for RL which was mainly associated to PC2 (r = 0.54), all the remaining traits displayed relatively high loading values (r) (absolute values > 0.54) with PC1. Under P deficiency, PCA analysis showed that, the first two PC (Eigenvalues of 12.64 and 2.54, respectively) accounted for 71.15% of the total variation (62.61% and 8.54%, respectively). Almost all the analyzed parameters were positively correlated with PC1 (r > 0.54), except for RL, RDW and RPC which displayed a high negative association with this axis (r < - 80). The 2D PCA plots showing the pattern of morpho-physiological variation of the studied populations in control conditions

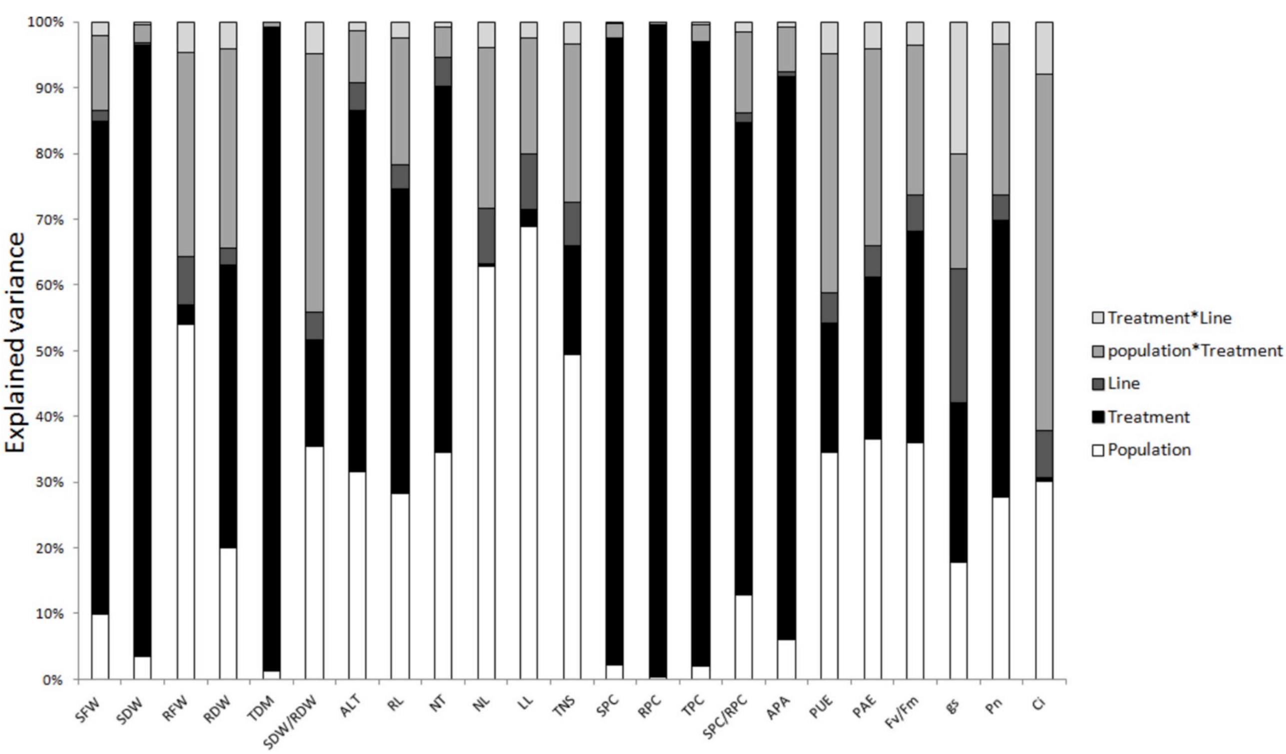

Fig. 2. Contribution of population, line, treatment factors and their interactions in the total explained variance for the analyzed traits.

Table 3
Pearson correlations between all the studied traits in control conditions (1 mM P) (below the diagonal) and under Low phosphorus availability (5 μM) (below and above the diagonal, respectively). Values in bold are different from 0 with a significance level alpha = 0.05.

Traits	SFW	SDW	RFW	RDW	TDM	SDW/RDW	ALT	RL	NT	NL	ALL	TNS	SPC	RPC	PPC	SPC/RPC	PAC	PUE	PAE	Fv/Fm	gs	Pn	Ci
SFW	1.00	0.09	0.12	0.19	0.17	−0.16	−0.04	−0.08	−0.02	−0.38	0.10	−0.29	0.04	−0.11	0.01	0.02	0.20	0.11	−0.17	0.44	−0.16	−0.22	−0.07
SDW	0.89	1.00	0.28	0.22	0.82	0.11	0.05	0.30	0.07	0.24	0.17	0.06	0.37	0.04	0.34	−0.01	−0.05	−0.03	0.16	0.32	−0.38	0.02	−0.24
RFW	0.60	0.56	1.00	0.72	0.62	−0.61	0.56	0.47	0.60	0.29	0.49	−0.39	0.52	0.52	0.57	0.07	−0.15	−0.35	0.00	0.30	−0.23	0.10	−0.35
RDW	−0.73	−0.86	−0.46	1.00	0.74	−0.91	0.21	0.25	0.25	0.06	0.21	−0.23	0.22	0.42	0.29	0.06	−0.20	−0.02	−0.47	0.19	−0.11	−0.04	−0.08
TDM	0.90	0.99	0.56	−0.79	1.00	−0.45	0.16	0.36	0.19	0.20	0.24	−0.09	0.39	0.27	0.40	0.03	−0.15	−0.03	−0.16	0.33	−0.33	−0.01	−0.22
SDW/RDW	0.85	0.96	0.53	−0.87	0.94	1.00	−0.18	−0.18	−0.16	0.00	−0.14	0.23	−0.14	−0.36	−0.20	−0.05	0.17	0.05	0.50	−0.08	0.02	0.01	−0.02
ALT	0.64	0.56	0.55	−0.49	0.55	0.59	1.00	0.54	0.87	0.22	0.81	−0.70	0.69	0.69	0.76	0.26	−0.16	−0.76	0.54	−0.04	−0.31	0.00	−0.39
RL	−0.93	−0.91	−0.59	0.77	−0.91	−0.85	−0.56	1.00	0.51	0.67	0.57	−0.40	0.67	0.36	0.67	0.20	−0.18	−0.59	0.44	0.22	−0.40	0.37	−0.37
NT	0.86	0.85	0.51	−0.71	0.85	0.84	0.57	−0.81	1.00	0.13	0.82	−0.69	0.63	0.70	0.70	0.34	−0.09	−0.68	0.45	0.02	−0.27	−0.02	−0.53
NL	0.85	0.85	0.50	−0.75	0.85	0.81	0.48	−0.83	0.82	1.00	0.17	0.12	0.40	0.11	0.38	0.06	−0.14	−0.34	0.34	0.18	−0.22	0.48	−0.23
ALL	0.84	0.96	0.53	−0.82	0.95	0.93	0.57	−0.88	0.78	0.79	1.00	−0.71	0.70	0.58	0.74	0.37	−0.09	−0.72	0.51	0.08	−0.26	0.11	−0.33
TNS	0.83	0.90	0.34	−0.75	0.90	0.85	0.44	−0.82	0.80	0.82	0.87	1.00	−0.37	−0.56	−0.45	−0.20	−0.03	0.46	−0.22	0.06	0.32	0.19	0.29
SPC	0.93	0.96	0.64	−0.81	0.96	0.91	0.62	−0.94	0.87	0.87	0.92	0.86	1.00	0.45	0.98	0.35	−0.15	−0.90	0.73	0.20	−0.33	0.24	−0.30
RPC	−0.57	−0.65	−0.50	0.53	−0.66	−0.62	−0.27	0.65	−0.59	−0.50	−0.65	−0.52	−0.65	1.00	0.62	0.09	−0.05	−0.59	0.27	−0.12	0.03	−0.09	−0.18
PPC	0.93	0.94	0.61	−0.80	0.93	0.89	0.64	−0.91	0.85	0.87	0.89	0.86	0.98	−0.51	1.00	0.33	−0.14	−0.92	0.70	0.15	−0.28	0.19	−0.30
SPC/RPC	0.92	0.95	0.65	−0.80	0.95	0.90	0.59	−0.94	0.86	0.84	0.92	0.85	0.99	−0.75	0.95	1.00	−0.03	−0.36	0.25	−0.03	−0.13	0.12	−0.13
PAC	0.90	0.93	0.64	−0.80	0.93	0.90	0.59	−0.91	0.82	0.83	0.90	0.85	0.94	−0.63	0.92	0.94	1.00	0.11	−0.01	0.02	−0.01	−0.14	0.07
PUE	0.54	0.75	0.28	−0.49	0.78	0.70	0.29	−0.59	0.56	0.52	0.72	0.67	0.59	−0.69	0.51	0.64	0.62	1.00	−0.82	0.01	0.15	−0.24	0.19
PAE	0.82	0.93	0.54	−0.95	0.89	0.93	0.60	−0.84	0.79	0.83	0.91	0.82	0.92	−0.54	0.92	0.90	0.88	0.54	1.00	−0.01	−0.17	0.20	−0.20
Fv/Fm	0.51	0.56	0.27	−0.51	0.55	0.60	0.48	−0.54	0.52	0.47	0.61	0.54	0.54	−0.36	0.52	0.53	0.50	0.39	0.56	1.00	−0.34	0.33	−0.24
gs	0.76	0.87	0.45	−0.78	0.86	0.87	0.50	−0.78	0.68	0.77	0.88	0.80	0.82	−0.58	0.80	0.82	0.78	0.66	0.84	0.57	1.00	0.00	0.64
Pn	0.76	0.84	0.53	−0.73	0.84	0.83	0.45	−0.77	0.69	0.76	0.85	0.82	0.82	−0.61	0.79	0.82	0.83	0.63	0.79	0.43	0.84	1.00	−0.11
Ci	0.69	0.80	0.35	−0.68	0.79	0.76	0.34	−0.74	0.69	0.70	0.76	0.76	0.76	−0.53	0.74	0.76	0.68	0.62	0.73	0.43	0.77	0.68	1.00

and under P deficiency on the basis of the 23 analyzed parameters were shown in Fig. 3A and Fig. 3B, respectively. In control conditions, the studied lines were divided into two major groups along PC1. The lines originated from Raouad and Sejnen were aggregated in a first small group in the negative side of PC1, whereas the lines of all the remaining populations seem to be in an extensive overlap in a second large group around the origin and in the positive side of PC1 (Fig. 3A). Under P deficiency (Fig. 3B), the studied lines were divided in two major groups clearly separated by the origin of PC1. The lines originated from Douar El Haj Wniss, El Kef, Fayedh, and Jbal Zaghouan were tendentiously discriminated from the remaining lines in the negative side of PC1 and seem to be very susceptible to P deficiency. This group of lines were distinguished by a stunted shoot growth, a considerable root biomass and a high P accumulation in roots. The lines of the remaining populations formed together a second group in the positive side of PC1. These groups of lines were characterized by an important aboveground biomass production, noticeable phosphorus use efficiency (PUE) and phosphorus acquisition efficiency (PAE). The latter could be divided into two subgroups. The first subgroup formed by the lines originated from Raouad and Sjenen and could be considered as tolerant to P deficiency, whereas the second subgroup, which enclosed the lines originated from Haouria and Enfidha, could be considered as moderately tolerant. Noticeably, the lines were found very close to each other and/or in extensive overlap in each of the defined groups, which indicate the weak within-populations genetic variation for adaptability to P-limiting conditions and suggests that the response to P deficiency was population-specific rather than line-specific. The pattern of lines grouping depicted by PCA analysis was supported by the hierarchical clustering analysis (HCA). In control conditions, two major groups of lines were identified (Fig. 4A). The first group enclosed the lines originated from Raouad and Sejnen and some lines from Jbal Zaghouan (Cluster 1A). Although displaying a moderate biomass production, this group of lines were distinguished by a considerable vigor and a high phosphorus acquisition efficiency (PAE) in control conditions in comparison with the other lines. The remaining lines, which were characterized by a relatively better biomass production, were grouped together in a second large cluster (Cluster 2A). Moreover, the same groups of tolerant (Cluster 2B), moderately tolerant (Cluster 1B) and susceptible (Cluster 3B) lines previously identified by PCA analysis were clearly depicted in the heat map showing the grouping of lines under P deficiency (Fig. 4B). Interestingly, the results of PCA and HCA revealed an overall better performance for the lines originated from Raouad and Sejnen under control and P deficiency conditions on the basis of the majority of studied parameters, suggesting their usefulness in future breeding programs.

3.4. Phosphorus responsiveness and efficiency

In order to investigate with more details the difference between the studied populations in terms of responsiveness and P efficiency, we analyzed REP, PSF, PAE and PUE as a function of the total of dry matter (TDM) produced at low P level and the lines were categorized as efficient responsive (ER), efficient non-responsive (ENR), non-efficient responsive (NER) and non-efficient non-responsive (NENR). The four ordination plots revealed similar patterns of populations grouping comparable to that showed by PCA and hierarchical clustering analyses. As shown in Fig. 5, all lines originated from Sjenen and Raouad fell into the group ER. Under P starvation, these group of lines produced a considerable dry matter yield (>1.77 g) and exhibited relatively high PAE and PUE values, ranging from 8.24 to 13.69 and from 0.38 to 0.48, respectively (Fig. 5A and B). These lines were also characterized by relatively low PSF values ranging from 21.51% to 36.2% and high REP levels varying between 65.15% and 78.48% (Fig. 5C and D). Meanwhile, except for few lines classified as NER and/or ENR, most of Enfidha and Haouria lines were considered as ER. This group of lines produced relatively lower dry matter and displayed slightly lower PAE,

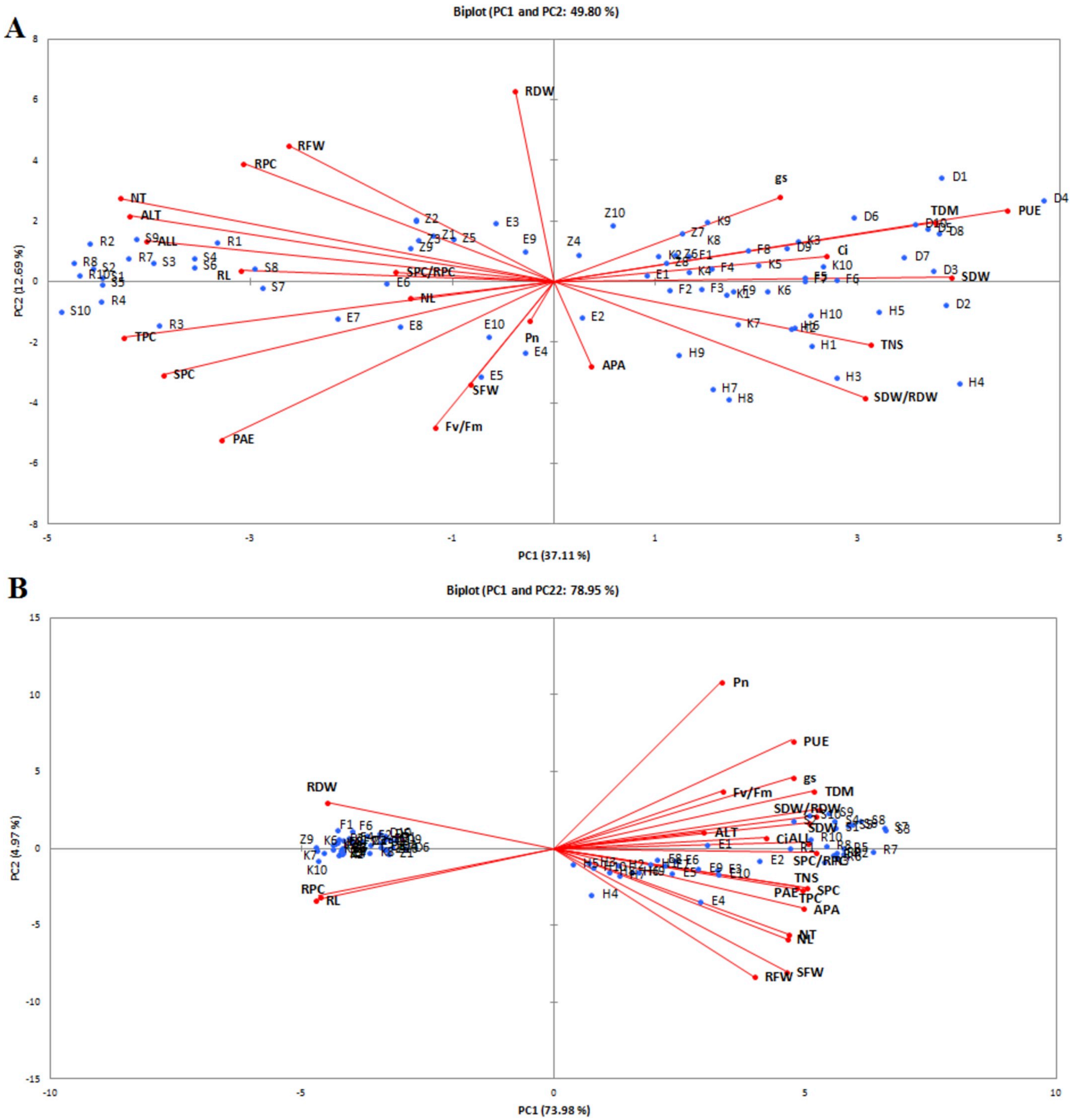

Fig. 3. Two-dimensional PCA plot showing the relationships among 79 lines representing the 8 populations of *Brachypodium hybridum*. A: Control conditions; B: under low P availability conditions.

PUE, PSF and REP under P-limiting conditions in comparison with those originated from Sjenen and Raouad. In contrast, all the lines originated from Douar El Haj wniss, El Kef, and Jbal Zaghouan were categorized as NENR as they exhibited a very low biomass production (< 1.2 g) and displayed relatively low PAE and PUE values (< 6.52 and < 0.33, respectively), a high relative reduction in dry matter production (PSF values > 50%) and a relatively low REP, ranging from 37% to 48% under P-limiting conditions. Similar to PCA and HCA results, these results confirmed that the response to P deficiency in the Tunisian *B. hybridum* collection was population-specific and support the usefulness of Sjenen and Raouad lines in future breeding programs.

4. Discussion

The analysis of morpho-physiological variation among seedlings exposed to early stress in controlled conditions has been considered a reliable approach for screening the plants stress tolerance and a crucial step for establishing future breeding program (Duangpan et al., 2018).

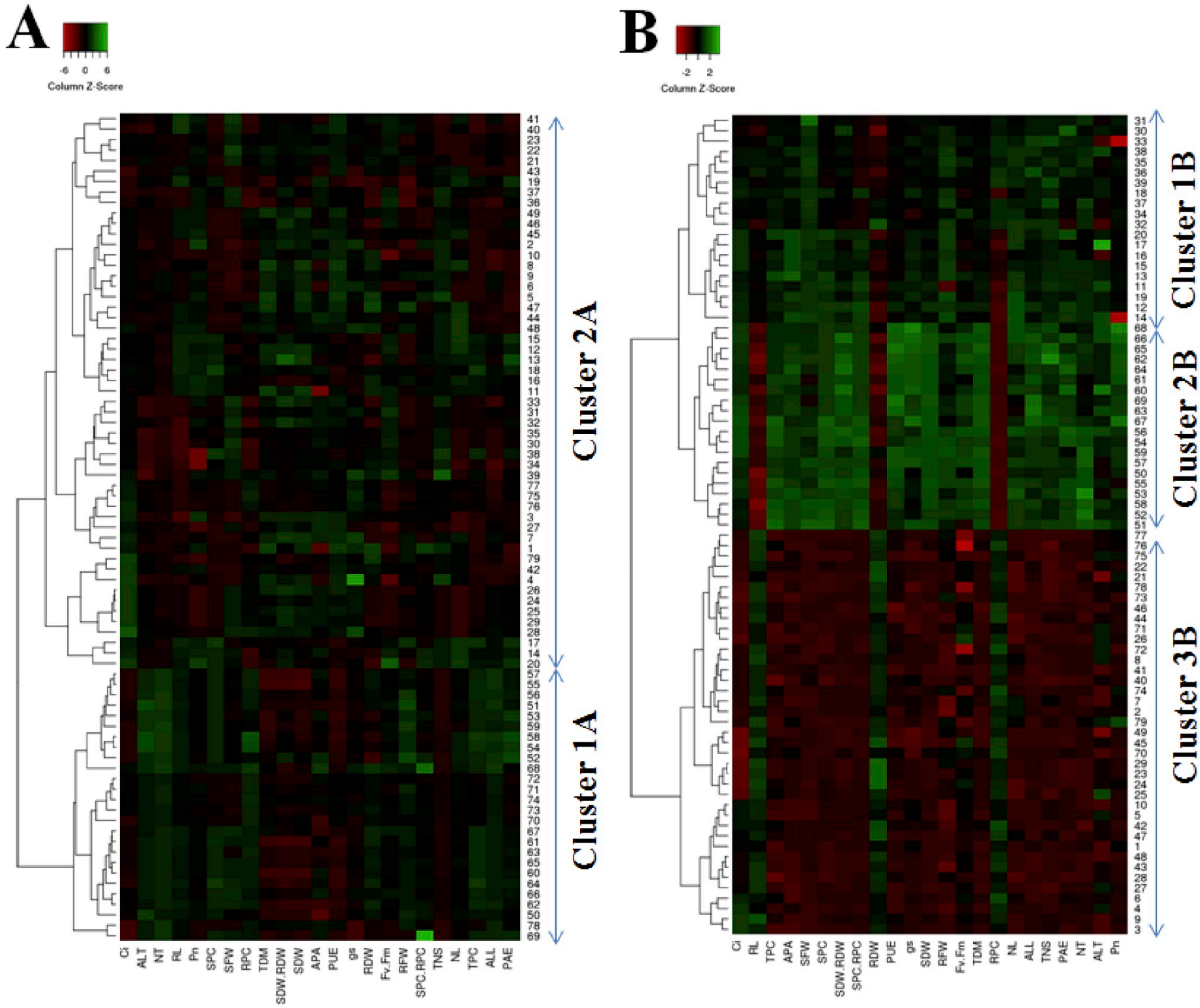

Fig. 4. Hierarchical clustering for the 79 *Brachypodium hybridum* lines in control conditions (A) and under low phosphorus availability (B). (Douar El Haj Wniss: 1 to 10; Enfidha: 11 to 20; Fayedh: 21 to 29; Haouria: 30 to 39; El Kef: 40 to 49; Raouad: 50 to 59; Sjenen: 60 to 69; Jbal Zaghouan: 70 to 79).

Phosphorus deficiency is a major abiotic stress that affects plant growth and productivity in both agricultural and natural ecosystems (Zhang et al., 2014). Previous studies have demonstrated that the response to P deficiency varies greatly between species as well as among genotypes within the same species (Bilal et al., 2018). In the present study, we analyzed the genetic variation of 79 diverse lines representing 8 Tunisian populations of *B. hybridum* issued after two generations of a spontaneous selfing using a large set of morpho-physiological parameters in order to screen their tolerance to P deficiency. Consistent with an earlier work realized on the parental populations of the same lines based on morpho-phenologial traits (Neji et al., 2015a), our results revealed a considerable and significant morpho-physiological variation in the studied *B. hybridum* collection in control and under P deficiency conditions. Noticeably, we recorded much higher variation under low P availability for most of analyzed parameters, which is in agreement with the study of Da Silveira et al. (2014) on sugarcane genotypes using agronomic traits. The same authors suggested that environmental stresses, including low nutrient availability, lead to an increased genetic variation because each genotype may displays a particular response to stress. However, in comparison with the results of Neji et al. (2015a), our results highlighted a relatively greater variation among populations than within populations in both studied treatments. We speculate that this pattern is likely due to the high selfing rate of this species and probably resulting from an extensive natural selection driving the genetic structure of the Tunisian populations of *B. hybridum* (Neji et al., 2015b). In agreement, Noël et al. (2017) have demonstrated that, during selection, selfing species experience a rapid decrease within-population phenotypic variance. Nevertheless, the results of the present study were in agreement with those obtained by Martínez et al. (2018) in Iberian populations of *B. hybridum* in Spain exposed to drought stress. The great morphological variation among populations could be also explained by the wide geographical distribution of *B. hybridum* in Tunisia, which may engender a considerable shifts in morpho-physiological traits along an environmental gradient. Besides, the coefficient of variation for all measured parameters under P deficiency were higher than their estimates under the normal conditions, suggesting that P deficiency may promote the genetic variation among *B. hybridum* populations for analyzed traits.

Moreover, for all analyzed traits, ANOVA revealed that the morpho-physiological variation was mainly explained by the treatment, population and their interaction. This result indicates that the variation of Tunisian populations of *B. hybridum* was highly influenced by P deficiency. Interestingly, the significant population*treatment interaction pointed towards an adaptive differential response in the studied populations. In agreement, a pattern of adaptive differential response to P deficiency was evidenced in diverse plants species (Baker, 1978).

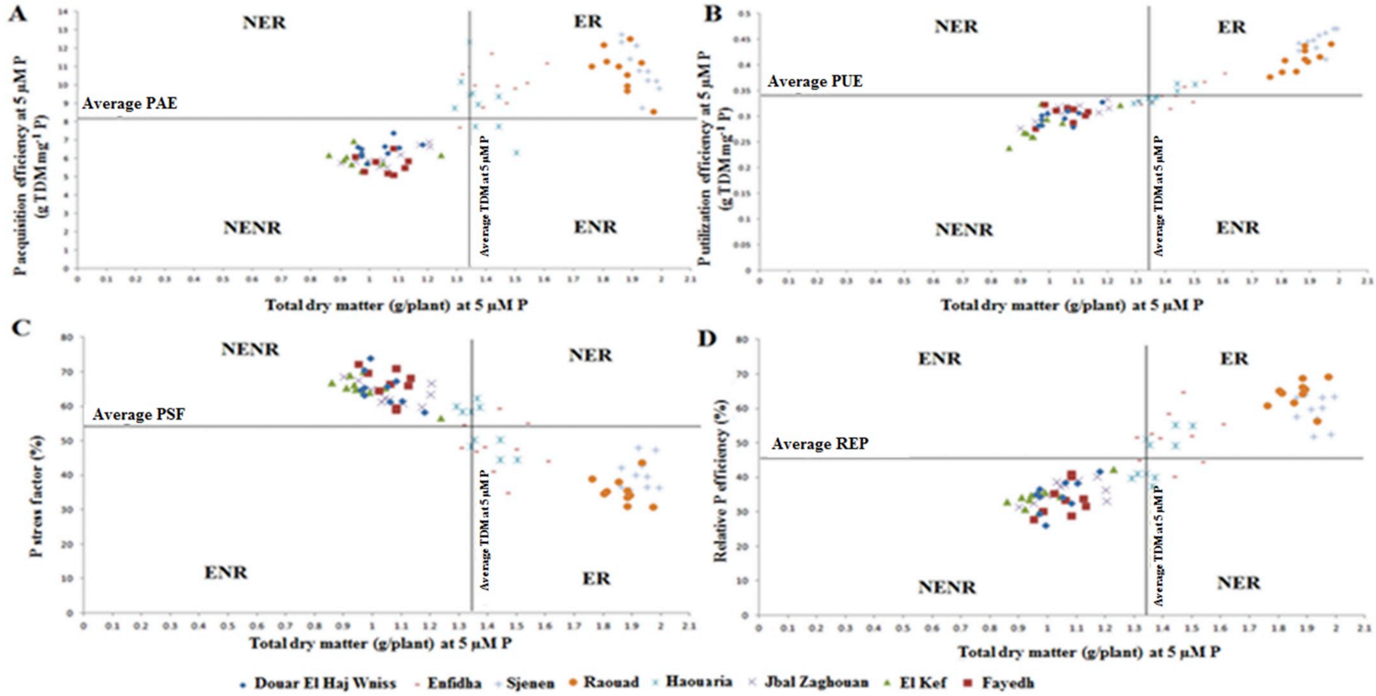

Fig. 5. Ordination plots to classify *Brachypodium hybridum* lines for total dry matter under low P availability as function of (A) P acquisitation effeciency, (B) P utilization efficiency (PUE), (C) P stress factor (PSF) and relative P efficiency. ER, efficient and responsive; NER, non-efficient but responsive; ENR, efficient but non-responsive; NENR, non-efficient and non-responsive.

Similar pattern was also observed in natural Spanish populations of *B. hybridum* populations (Manzaneda et al., 2015; Martínez et al., 2018) and its diploid progenitor *B. distachyon* (Manzaneda et al., 2015) in response to drought stress. In accordance, our results suggest that morpho-physiology of *B. hybridum* in its native range varies greatly with both environmental (climate) and edaphic conditions (nutriment availability and soil type) of the populations' origin.

Heritability provides insights about the relative contribution of the genetic and environmental factors in governing the genetic variation of a given trait and the extent of its transmission into future generations, and thus it is very helpful in determining a genotype/line's usefulness for the future selection and improvement schemes (Mazid et al., 2013). Our results revealed relatively high levels of heritability for most of analyzed traits in both control and under P deficiency, suggesting that the morpho-physiological variation of *B. hybridum* in Tunisia was under a strong genetic control. Noteworthy, our results revealed very high heritability for the morphological and PE related traits in both studied conditions, with slightly higher values recorded under P deficiency. According to Gemenet et al. (2016), this pattern suggests that the direct selection of these agriculturally-relevant traits via a direct selection under P deficiency would be more effective than the indirect one under optimal P supply. In accordance with our results, high heritability estimates for growth and PE related traits were recorded in diverse plants species (Ao et al., 2010). In contrast, we noticed a relatively low heritability for the photosynthetic parameters in control and under low P availability, which suggest that the variation of these traits was highly influenced by environment, and thus their inutility in future breeding programs. This result was somewhat expected as the Tunisian *B. hybridum* populations were originated from areas differing greatly in their bioclimatic conditions since photosynthesis is highly influenced by environmental factors (Gururani et al., 2015). Thus, we speculate that each population develops a photosynthetic process fitting with its local environment.

Furthermore, we detected strong positive correlations between all traits related to belowground biomass allocation, those related to photosynthetic process as well as the PE related traits under P deficiency conditions. This result might be very useful for breeders to easily select lines of both high yield and suitable P efficiency. In accordance with our results, many previous studies reporting negative correlations between biomass production and P concentration have suggested that the selection efforts may provide undesirable low yield lines (Missaoui and Young, 2016). Furthermore, similar to many previous studies (Ao et al., 2010), our results revealed as negative correlation between roots related traits and the other traits under P deficiency conditions. Interestingly, despite the significant increase in root length under limiting P conditions, we noticed a small difference in root biomass production between the optimal P level and the P limiting conditions. Such pattern suggests a contrasted biomass partitioning (shoots vs roots) and a preferential phosphorus allocation in shoots under low P availability, which could be a strategy to cope with extreme low P availability (Ao et al., 2010).

Moreover, our results revealed a high level of inter-populations morpho-physiological differentiation (Qst) under control and P deficiency on the basis of analyzed traits, indicating that the response to P deficiency in *B. hybridum* was most likely population-specific. Interestingly, the Qst values of all analyzed traits under both P levels were found strongly higher than the level of genetic differentiation among the Tunisian populations *B. hybridum* detected using SSR ($\Phi_{PT} = 0.023$) and insertion–deletion markers (InDel) ($\Phi_{PT} = 0.2$), which indicates an obvious adaptive differentiation of the analyzed traits and confirms the adaptive differential response in *B. hybridum* populations in response to P deficiency emphasized by ANOVA results. Similar pattern of adaptive response has been previously highlighted in natural populations of *Brachypodium* species, including *B. distachyon* (Dell'Acqua et al., 2014) as well as other plant species belonging to diverse families such as *Leymus chinensis* (Poaceae) (Yuan et al., 2016) and *Antirrhinum majus* (Plantaginaceae) (Mousset et al., 2018).

Furthermore, Previous studies have demonstrated that *B. distachyon* complex (*B. distachyon*, *B. stacei* and *B. hybridum*) displayed a wide adaptation to different environmental conditions across their Mediterranean native geographical ranges, which allow them to tolerate various biotic and abiotic constraints (Lopez-Alvarez et al., 2015;

López-Alvarez et al., 2012; Marques et al., 2017). However, with a geographical distribution largely exceeding those of its two progenitors, *B. hybridum* seems to be more adapted to various selective pressures, including drought, altitude, nutriments availability, and temperature (López-Alvarez et al., 2012; Marques et al., 2017). Similarly, (Neji et al. (2015a) have reported a wide geographical distribution of *B. hybridum* across various ecological and bioclimatic conditions, suggesting that the Tunisian populations of this species display diverse phenotypic and physiological adaptations to environmental stresses. In the present study, PCA and hierarchical clustering showed that lines of the same population were grouped together in both control and P deficiency conditions, confirming the low within-populations variation. Interestingly, although populations were not grouped according to their geographical distribution, which is in accordance with the findings of Neji et al. (2015a), a different pattern of populations grouping was observed in this study. This was most likely due to the difference in analyzed parameters, which were mainly related to plant morphology in the previous study, whereas a large set of morpholo-physiological were analyzed in the current work. In agreement, by using different SNP sets, Chao et al. (2010) have explained the difference of clustering pattern in *Triticum aestivum* L. populations by an extensive selection for adaptation to various environmental conditions.

Many previous studies have demonstrated the reliability of PCA and hierarchical clustering in identifying P-efficient genotypes on the basis of diverse morpho-physiological traits (Ozturk et al., 2005; Pan et al., 2008). Additionally, a multitude of investigations have attributed the Phosphorus efficiency of given a genotype to diverse morpho-physiological features resulting from its ability to mobilize P from the soil to its areal parts (P uptake) or to utilize P for maintaining vigorous growth and producing high biomass under P deficiency (utilization efficiency) (Balemi and Schenk, 2009). Our PCA and hierarchical clustering analyses, with a strong correlation between the traits related to plant vigor, biomass production and P related traits, clearly disclosed two non-overlapping groups of lines; P efficient (Raouad and Sejnen) and moderately P efficient (Haouria and Enfidha) characterized by relatively a small decrease in their aboveground growth, biomass production under P deficiency conditions. In contrast, the remaining populations, which showed distinctive signs of latent P deficiency, including reduced tillering, a striking decrease in biomass production and increased shoot to root ratio (Frydenvang et al., 2015), were grouped together and considered as susceptible to P deficiency. Noticeably, our results revealed that, under P deficiency, the susceptible lines showed a stronger root proliferation than the tolerant and the moderately tolerant ones. However, a contrasted pattern was recorded for acid phosphatase activity, with higher increase in APA revealed in the tolerant lines. Although many previous studies have demonstrated that the significant root proliferation under low P availability is a strategy of enhancing P acquisition (Ramaekers et al., 2010; Vejchasarn et al., 2016), Zhao et al. (2018) suggested that root length and biomass play a minor role in improving P uptake in transgenic *B. distachyon* lines. Given the importance of APA in enhancing low-phosphorus tolerance in crops (López-Arredondo et al., 2014), we speculate that increased APA play a major role in promoting the tolerance of *B. hybridum* to P starvation. Importantly, Akhtar et al. (2008) have considered the biomass production under low P availability as a determinant agricultural parameter for categorizing low P-tolerant plants in terms of efficiency and responsiveness. For breeding and selection purposes, Neto et al. (2016) have proposed to categorize available germplasm with regard P efficiency and responsiveness in four groups: the 'Efficient-Responsive' (ER), with a high biomass production under low-P availability and a positive response to P fertilization; (ii) non-efficient responsive (NER), with a low biomass production under limiting P conditions and a positive response to P fertilization; (iii) efficient nonresponsive (ENR), with a high production of dry mass under low-P conditions but without a response to P fertilization; and (iv) non-efficient nonresponsive (NENR), with a low biomass production under low-P conditions and no response to P fertilization. According to (Akhtar et al., 2008), ER group is the most enviable for breeding, followed by ENR group, whereas the NENR group was considered the undesirable. Obviously, our results disclosed three non-overlapping groups of efficiency and responsiveness associated with population structure and extensively correlated with environmental conditions. Lines inhabiting coastal populations which were classified as ER (Raouad and Sejnen) or a combination of ER and ENR (Enfidha and Haouria). Conversely, all the remaining lines which were originated from inland populations were classified as NENR group. This classification pattern suggests the performance to P deficiency in *B. hybridum* is mainly associated with geographical variables, reflecting climatic and edaphic factors related soil type, texture, chemistry and moisture. Importantly, according to Akhtar et al. (2008), our results indicated that the lines originated from the coastal populations (Raouad, Sejnen, Haouria and Enfidha) appear to be good candidates for future studies and breeding programs.

Authors contribution

Mohamed Neji contributed in the experiment design, analyzed the data and wrote the manuscript. Saber Kouas contributed in the experiment design and assisted the experimental work. Mhemmed Gandour assisted in the preparation of the plant material. Samir Aydi contributed in some experimental activities. Chedly Abdelly assisted in supervising the work.

Acknowledgements

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.006.

Conflicts of interest

All the authors agree that there was no conflict of interest to declare.

References

Akhtar, M.S., Oki, Y., Adachi, T., 2008. Intraspecific variations of phosphorus absorption and remobilization, P forms, and their internal buffering in *Brassica* cultivars exposed to a P-stressed environment. J. Integr. Plant Biol. 50, 703–716. https://doi.org/10.1111/j.1744-7909.2008.00675.x.

Ao, J., Fu, J., Tian, J., Yan, X., Liao, H., 2010. Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. Funct. Plant Biol. 37, 304. https://doi.org/10.1071/FP09215.

Baker, A.J.M., 1978. Zinc-phosphorus interactions IN a zinc-tolerant and a NON-tolerant population OF silene maritima with. New Phytol. 81, 331–339. https://doi.org/10.1111/j.1469-8137.1978.tb02638.x.

Balemi, T., Schenk, M.K., 2009. Genotypic variation of potato for phosphorus efficiency and quantification of phosphorus uptake with respect to root characteristics. J. Plant Nutr. Soil Sci. 172, 669–677. https://doi.org/10.1002/jpln.200800246.

Bilal, H.M., Aziz, T., Maqsood, M.A., Farooq, M., Yan, G., 2018. Categorization of wheat genotypes for phosphorus efficiency. PLoS One 13, e0205471. https://doi.org/10.1371/journal.pone.0205471.

Chao, S., Dubcovsky, J., Dvorak, J., Luo, M.-C., Baenziger, S.P., Matnyazov, R., Clark, D.R., Talbert, L.E., Anderson, J.A., Dreisigacker, S., Glover, K., Chen, J., Campbell, K., Bruckner, P.L., Rudd, J.C., Haley, S., Carver, B.F., Perry, S., Sorrells, M.E., Akhunov, E.D., 2010. Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (Triticum aestivum L.) BMC Genomics 11, 727. https://doi.org/10.1186/1471-2164-11-727.

Da Silveira, G., de A. Costa, P.M., Kist, V., de F. Almeida, C., Baffa, D.C.F., Barbosa, M.H.P., 2014. Genetic variation affecting agronomic traits in sugarcane in response to high and low phosphorus availability. Agron. J. 106, 2296. https://doi.org/10.2134/agronj14.0199.

Dell'Acqua, M., Zuccolo, A., Tuna, M., Gianfranceschi, L., Pè, M., 2014. Targeting environmental adaptation in the monocot model *Brachypodium distachyon*: a multi-faceted approach. BMC Genomics 15, 801. https://doi.org/10.1186/1471-2164-15-801.

Duangpan, S., Buapet, P., Sujitto, S., Eksomtramage, T., 2018. Early assessment of

drought tolerance in oil palm D × P progenies using growth and physiological characters in seedling stage. Plant Genet. Resour. Charact. Util. 16, 544–554. https://doi.org/10.1017/S1479262118000151.
Fleury, P., Leclerc, M., 1943. Dosage colorimétrique du phosphore urinaire par la méthode nitrovanadomolybdique de Misson.
Frydenvang, J., van Maarschalkerweerd, M., Carstensen, A., Mundus, S., Schmidt, S.B., Pedas, P.R., Laursen, K.H., Schjoerring, J.K., Husted, S., 2015. Sensitive detection of phosphorus deficiency in plants using chlorophyll *a* fluorescence. Plant Physiol. 169, 353–361. https://doi.org/10.1104/pp.15.00823.
Gemenet, D.C., Leiser, W.L., Beggi, F., Herrmann, L.H., Vadez, V., Rattunde, H.F.W., Weltzien, E., Hash, C.T., Buerkert, A., Haussmann, B.I.G., 2016. Overcoming phosphorus deficiency in west african pearl millet and sorghum production systems: promising options for crop improvement. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.01389.
Gururani, M.A., Venkatesh, J., Tran, L.S.P., 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. Mol. Plant 8, 1304–1320. https://doi.org/10.1016/j.molp.2015.05.005.
Kim, D.Y., Hong, M.J., Jang, J.H., Seo, Y.W., 2012. cDNA-AFLP analysis reveals differential gene expression in response to salt stress in Brachypodium distachyon. Genes Genomics 34, 475–484. https://doi.org/10.1007/s13258-012-0067-z.
Kouas, S., Louche, J., Debez, A., Plassard, C., Drevon, J.J., Abdelly, C., 2009. Effect of phosphorus deficiency on acid phosphatase and phytase activities in common bean (Phaseolus vulgaris L.) under symbiotic nitrogen fixation. Symbiosis 47 (3), 141–149. https://doi.org/10.1007/BF03179974.
López-Alvarez, D., López-Herranz, M.L., Betekhtin, A., Catalán, P., 2012. A DNA barcoding method to discriminate between the model plant *Brachypodium distachyon* and its close relatives B. Stacei and B. Hybridum (Poaceae). PLoS One 7, e51058. https://doi.org/10.1371/journal.pone.0051058.
Lopez-Alvarez, D., Manzaneda, A.J., Rey, P.J., Giraldo, P., Benavente, E., Allainguillaume, J., Mur, L., Caicedo, A.L., Hazen, S.P., Breiman, A., Ezrati, S., Catalan, P., 2015. Environmental niche variation and evolutionary diversification of the Brachypodium distachyon grass complex species in their native circum-Mediterranean range. Am. J. Bot. 102, 1073–1088. https://doi.org/10.3732/ajb.1500128.
López-Arredondo, D.L., Leyva-González, M.A., González-Morales, S.I., López-Bucio, J., Herrera-Estrella, L., 2014. Phosphate nutrition: improving low-phosphate tolerance in crops. Annu. Rev. Plant Biol. 65, 95–123. https://doi.org/10.1146/annurev-arplant-050213-035949.
Manzaneda, A.J., Rey, P.J., Anderson, J.T., Raskin, E., Weiss-Lehman, C., Mitchell-Olds, T., 2015. Natural variation, differentiation, and genetic trade-offs of ecophysiological traits in response to water limitation in *Brachypodium distachyon* and its descendent allotetraploid *B. hybridum* (Poaceae): ploidy and adaptation in *Brachypodium distachyon*. Evolution 69, 2689–2704. https://doi.org/10.1111/evo.12776.
Manzaneda, A.J., Rey, P.J., Bastida, J.M., Weiss-Lehman, C., Raskin, E., Mitchell-Olds, T., 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in Brachypodium distachyon (Poaceae). New Phytol. 193, 797–805. https://doi.org/10.1111/j.1469-8137.2011.03988.x.
Marques, I., Shiposha, V., López-Alvarez, D., Manzaneda, A.J., Hernandez, P., Olonova, M., Catalán, P., 2017. Environmental isolation explains Iberian genetic diversity in the highly homozygous model grass *Brachypodium distachyon*. BMC Evol. Biol. 17. https://doi.org/10.1186/s12862-017-0996-x.
Martínez, L.M., Fernández-Ocaña, A., Rey, P.J., Salido, T., Amil-Ruiz, F., Manzaneda, A.J., 2018. Variation in functional responses to water stress and differentiation between natural allopolyploid populations in the *Brachypodium distachyon* species complex. Ann. Bot. 121, 1369–1382. https://doi.org/10.1093/aob/mcy037.
Mazid, M.S., Rafii, M.Y., Hanafi, M.M., Rahim, H.A., Shabanimofrad, M., Latif, M.A., 2013. Agro-morphological characterization and assessment of variability, heritability, genetic advance and divergence in bacterial blight resistant rice genotypes. South Afr. J. Bot. 86, 15–22. https://doi.org/10.1016/j.sajb.2013.01.004.
Missaoui, A.M., Young, J., 2016. Genetic gain from selection and potential for improving alfalfa phosphorus uptake and removal from soils heavily amended with poultry litter. Euphytica 209, 495–506. https://doi.org/10.1007/s10681-016-1677-3.
Mousset, M., Marin, S., Archambeau, J., Blot, C., Bonhomme, V., Garaud, L., Pujol, B., 2018. Genotype by environment interactions for the shade avoidance syndrome in the plant Antirrhinum majus, the snapdragon. bioRxiv 331033. https://doi.org/10.1101/331033.
Neji, Mohamed, Geuna, F., Taamalli, W., Ibrahim, Y., Chiozzotto, R., Abdelly, C., Gandour, M., 2015. Assessment of genetic diversity and population structure of Tunisian populations of Brachypodium hybridum by SSR markers. Flora - Morphol. Distrib. Funct. Ecol. Plants 216, 42–49. https://doi.org/10.1016/j.flora.2015.08.001.
Neji, M., Geuna, F., Taamalli, W., Ibrahim, Y., Smida, M., Badri, M., Abdelly, C., Gandour, M., 2015. Morpho-phenological diversity among Tunisian natural populations of *Brachypodium hybridum*. J. Agric. Sci. 153, 1006–1016. https://doi.org/10.1017/S0021859614000689.
Neto, A.P., Favarin, J.L., Hammond, J.P., Tezotto, T., Couto, H.T.Z., 2016. Analysis of phosphorus use efficiency traits in coffea genotypes reveals *Coffea arabica* and *Coffea canephora* have contrasting phosphorus uptake and utilization efficiencies. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.00408.
Noël, E., Jarne, P., Glémin, S., MacKenzie, A., Segard, A., Sarda, V., David, P., 2017. Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations. Curr. Biol. 27, 237–242. https://doi.org/10.1016/j.cub.2016.11.015.
Ozturk, L., Eker, S., Torun, B., Cakmak, I., 2005. Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. Plant Soil 269, 69–80. https://doi.org/10.1007/s11104-004-0469-z.
Pan, X., Li, W., Zhang, Q., Li, Y., Liu, M., 2008. Assessment on phosphorus efficiency characteristics of soybean genotypes in phosphorus-deficient soils. Agric. Sci. China 7, 958–969. https://doi.org/10.1016/S1671-2927(08)60135-2.
Pang, J., Zhao, H., Bansal, R., Bohuon, E., Lambers, H., Ryan, M.H., Siddique, K.H.M., 2018. Leaf transpiration plays a role in phosphorus acquisition among a large set of chickpea genotypes: leaf transpiration and P acquisition in chickpea. Plant Cell Environ. 41 (9), 2069–2079. https://doi.org/10.1111/pce.13139.
Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412.
Poiré, R., Chochois, V., Sirault, X.R.R., Vogel, J.P., Watt, M., Furbank, R.T., 2014. Digital imaging approaches for phenotyping whole plant nitrogen and phosphorus response in *Brachypodium distachyon*: digital imaging approaches for phenotyping. J. Integr. Plant Biol. 56, 781–796. https://doi.org/10.1111/jipb.12198.
Ramaekers, L., Remans, R., Rao, I.M., Blair, M.W., Vanderleyden, J., 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. Field Crop. Res. 117, 169–176. https://doi.org/10.1016/j.fcr.2010.03.001.
Sulieman, S., Tran, L.-S.P., 2015. Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. Plant Sci. 239, 36–43. https://doi.org/10.1016/j.plantsci.2015.06.018.
Vejchasarn, P., Lynch, J.P., Brown, K.M., 2016. Genetic variability in phosphorus responses of rice root phenotypes. Rice 9. https://doi.org/10.1186/s12284-016-0102-9.
Verelst, W., Bertolini, E., De Bodt, S., Vandepoele, K., Demeulenaere, M., Enrico Pè, M., Inzé, D., 2013. Molecular and physiological analysis of growth-limiting drought stress in Brachypodium distachyon leaves. Mol. Plant 6, 311–322. https://doi.org/10.1093/mp/sss098.
Vogel, J.P., Tuna, M., Budak, H., Huo, N., Gu, Y.Q., Steinwand, M.A., 2009. Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. BMC Plant Biol. 9, 88. https://doi.org/10.1186/1471-2229-9-88.
Wang, X., Shen, J., Liao, H., 2010. Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? Plant Sci. 179, 302–306. https://doi.org/10.1016/j.plantsci.2010.06.007.
Yuan, S., Guo, C., Ma, L., Wang, R., 2016. Environmental conditions and genetic differentiation: what drives the divergence of coexisting *Leymus chinensis* ecotypes in a large-scale longitudinal gradient? J. Plant Ecol. 9, 616–628. https://doi.org/10.1093/jpe/rtv084.
Zhang, J., Cruz De Carvalho, M.H., Torres-Jerez, I., Kang, Y., Allen, S.N., Huhman, D.V., Tang, Y., Murray, J., Sumner, L.W., Udvardi, M.K., 2014. Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. Plant Cell Environ. 37, 2553–2576.
Zhao, P., Wang, L., Yin, H., 2018. Transcriptional responses to phosphate starvation in *Brachypodium distachyon* roots. Plant Physiol. Biochem. 122, 113–120. https://doi.org/10.1016/j.plaphy.2017.11.010.