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## Embolism and mechanical resistances play a key-role in dehydration tolerance of a perennial grass *Dactylis glomerata* L

Florence Volaire, Frederic Lens, Hervé Cochard, Hueng Xu, Larissa Chacon Doria, Pauline Bristiel, Jennifer A. Balachowski, Nick P Rowe, Cyrille Violle, Catherine Picon-Cochard

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## **Embolism and mechanical resistances play a key-role in dehydration tolerance of a perennial grass *Dactylis glomerata* L.**

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1 **Embolism and mechanical resistances play a key-role in dehydration tolerance of a**  
2 **perennial grass *Dactylis glomerata* L.**

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25 **Running title:** Embolism resistance and dehydration tolerance in a perennial grass

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27 Original Article

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32

33 **Abstract**

34

35 • **Background and Aims** More intense droughts under climate change threaten species  
36 resilience. Hydraulic strategies determine drought survival in woody plants but have been hardly  
37 studied in herbaceous species. We explored the intra-specific variability of hydraulic and  
38 morphological traits as indicators of dehydration tolerance in a perennial grass, cocksfoot  
39 (*Dactylis glomerata*) which has a large biogeographical distribution in Europe.

40 • **Methods** Twelve populations of cocksfoot originating from Mediterranean, Temperate and  
41 Northern European areas were grown in a controlled environment in pots. Dehydration tolerance,  
42 leaf and stem anatomical traits and xylem pressure associated with 88% or 50% loss of xylem  
43 conductance ( $P_{88}$ ,  $P_{50}$ ) were measured.

44 • **Key Results** Across the twelve populations of cocksfoot,  $P_{50}$  ranged from -3.06 to -6.36 MPa,  
45 while  $P_{88}$  ranged from -5.06 to -11.6 MPa. This large intraspecific variability of embolism  
46 thresholds corresponded with the biogeographical distribution and some key traits of the  
47 populations. In particular,  $P_{88}$  was correlated with dehydration tolerance ( $r=-0.79$ ). The  
48 dehydration-sensitive temperate populations exhibited the highest  $P_{88}$  (-6.1 MPa). The most  
49 dehydration-tolerant Mediterranean populations had the greatest leaf dry matter content and leaf  
50 fracture toughness, and the lowest  $P_{88}$  (-10.4 MPa). The Northern populations displayed  
51 intermediate trait values, potentially attributable to frost resistance. The thickness of metaxylem  
52 vessel walls in stems was highly correlated with  $P_{50}$  ( $r=-0.92$ ) but no trade-off with stem  
53 lignification was observed. The relevance of the linkage between hydraulic and stomatal traits is  
54 discussed for drought survival in perennial grasses.

55 • **Conclusions** Compared to woody species, the large intra-specific variability in dehydration  
56 tolerance and embolism resistance within cocksfoot has consequences for its sensitivity to  
57 climate change. To better understand adaptive strategies of herbaceous species to increasing  
58 drought and frost requires exploring further the role of hydraulic and mechanical traits using a  
59 larger inter- and intra- specific range of species.

60

61 **Key-words:** cavitation resistance, *Dactylis glomerata* L., dehydration tolerance, drought survival,  
62 embolism threshold, plant mortality, leaf fracture toughness, vessel wall thickness, stem anatomy,  
63 intra-specific variability, perennial grass

## 64 INTRODUCTION

65 Higher temperatures due to climate change have led to more frequent extreme climate events  
66 around the world (I.P.C.C., 2014, Orlowsky and Seneviratne, 2012, Seneviratne et al., 2014).  
67 Extreme events, including intense droughts and heat waves, will become important drivers of  
68 future ecosystem dynamics and function (Smith, 2011). Resilience, defined as the capacity of a  
69 population or community to recover following disturbance, may therefore be the key to the  
70 persistence of ecosystems (Hodgson et al., 2015; Nimmo et al., 2015).

71 Forests and grasslands are major terrestrial ecosystems, covering one-quarter and one-  
72 third of the total land area, respectively. They provide many important ecosystem services  
73 including timber and forage supply, carbon storage, and biodiversity preservation, but their  
74 persistence is threatened under more frequent extreme climate events (Anderegg et al., 2013,  
75 Pilgrim et al., 2010). Climate-induced forest dieback is an increasing global concern (Allen et al.,  
76 2010) and major research efforts have been carried out to identify strategies for drought survival  
77 among woody plant species (Pivovarovoff et al., 2015). In parallel, declines in grassland  
78 productivity (Brookshire and Weaver, 2015) and long-term degradation from drought may  
79 gradually become more common in the future (Ciais et al., 2005). Drought reduces plant  
80 productivity and induces plant mortality in grasslands under extreme events (Griffin and  
81 Hoffmann, 2012, Hodgkinson and Muller, 2005, Moran et al., 2014, Poirier et al., 2012, Van Peer  
82 et al., 2001). Although the ability of woody species to survive intense drought was strongly  
83 related to their embolism resistance i.e. the vulnerability to embolism (McDowell et al., 2008),  
84 the traits and processes involved in the survival and recovery of herbaceous species after extreme  
85 stress are still poorly characterized (Craine et al., 2013, Hoover et al., 2014).

86 Recent methodological developments have enabled **inter-specific** study of the vulnerability to  
87 embolism in stems of herbaceous plants (Lens et al., 2013, Tixier et al., 2013), opening the door  
88 for explorations of the role of hydraulic traits in stress survival of non-woody species. Building  
89 on these recent results showing large interspecific variability in embolism resistance in grasses  
90 distributed along an aridity gradient (Lens et al., 2016), the present study aimed to explore the  
91 extent of intraspecific variability in the perennial grass *Dactylis glomerata* L. **This model species**  
92 **was chosen since it has a large biogeographical distribution over Europe and Asia (Borrill, 1991)**  
93 **and a high genetic variation within both populations and geographical origins (Lumaret, 1988,**  
94 **Xie et al., 2010).**

95 Intraspecific variation with respect to embolism resistance is still poorly understood  
96 (Martinez-Vilalta et al., 2009). In woody trees, phenotypic plasticity in resistance to embolism  
97 was found to be low and buffered against environmental variation in a large range of *Pinus*  
98 *pinaster* (Lamy et al., 2011, Lamy et al., 2014) but was not different across populations of *Fagus*  
99 *sylvatica* (Herbette et al., 2010, Wortemann et al., 2011); however, not a single study has  
100 assessed the intraspecific variation within an herbaceous grass species. Perennial species exhibit  
101 various functional strategies to cope with moderate to severe drought (Pérez-Ramos et al., 2013,  
102 Volaire, 2008, Zwicke et al., 2015), since they undergo many successive periods of stress and  
103 recovery during their life cycle. Under severe drought, the basal aerial meristems and roots are  
104 the key organs for plant survival and recovery, while most leaves of perennial grasses senesce to  
105 reduce water loss (Volaire et al., 2009). As a result, no significant correlation was found between  
106 vulnerability of leaf hydraulic conductance and whole-plant survival of drought in grasses  
107 (Ocheltree et al., 2016). The role of hydraulic traits in herbaceous species should therefore be  
108 analysed through a whole-plant perspective.

109 Survival in increasingly drought prone environments depends on a range of traits which include  
110 sufficient mechanical strengthening to avoid irreversible effects of tissue dehydration. Embolism  
111 resistant tree species are often characterized by a high wood density (Hacke et al., 2001, Lens et  
112 al., 2013, Ogasa et al., 2013) although no strong evidence was that denser woods conferred  
113 higher survival, or that the risk of embolism caused by wide conduits increased mortality (Russo  
114 et al., 2010). In addition, increased tolerance to hydraulic dysfunction implies increased carbon  
115 costs for leaf construction and water use (Nardini et al., 2012). Low embolism threshold values  
116 were associated with narrower and denser vein conduits, increased thickness of conduit wall and  
117 vein density and also with reduced leaf area (Nardini et al., 2012). In herbaceous species too,  
118 *Arabidopsis* plants with increased wood formation in their stems were shown to be significantly  
119 more embolism-resistant (Lens et al., 2013, Tixier et al., 2013) and grass species from the most  
120 arid environments had more lignified stems compared to the vulnerable species native to wetter  
121 habitats (Lens et al., 2016).

122 The main objective of this study is to identify relationships between embolism resistance, whole  
123 plant dehydration survival, leaf traits, and stem anatomy amongst twelve populations of *Dactylis*  
124 *glomerata* L originating from Scandinavia to Morocco. Given the contrasted biogeographic  
125 origins of the studied populations, we expected that they differ strongly for hydraulic and

126 anatomical traits which would subsequently contribute to their dehydration tolerance. To  
127 determine ecological responses of grassland species under climate extremes (Smith, 2011a), this  
128 study therefore aims to (a) identify the role of embolism thresholds and associated traits for  
129 drought survival and mechanical resistance in a perennial herbaceous species and (b) determine  
130 the intra-specific variability of these traits as indicators of potential drought survival.

131

## 132 MATERIAL AND METHODS

133

### 134 *Plant Material*

135 Twelve populations of *Dactylis glomerata* L. (cocksfoot), including native populations and  
136 cultivars, were selected from germplasm banks to be representative from three climatic regions:  
137 four Mediterranean, four Temperate, and four Northern populations (Table 1). Cocksfoot (*D.*  
138 *glomerata* L.) is a tetraploid ( $2n=4x=28$ ) species from the genus *Dactylis* whose taxonomic  
139 constitution is debated, but broadly consists of a complex of diploids and tetraploids (Borrill,  
140 1991). The twelve populations tested were all verified to be tetraploid (Barre, pers. comm).

141

### 142 *Experimental sites and design*

143 Three pot-experiments were carried out to determine dehydration tolerance, embolism resistance  
144 as well as anatomy and leaf traits, at the CEFÉ-CNRS research center in Montpellier, France  
145 (43°6'N, 3°8'E). Seeds from each *D. glomerata* population were sown in seedling trays in  
146 February 2013 and the adult plants (over one year old) were used for all subsequent experiment.

147 - Experiment 1 was carried out to determine plant dehydration tolerance measured as plant  
148 survival rate in plants growing in short pots, after rewatering following an intense drought  
149 (Volaire, 2018). Plants were transplanted into 4L, 18cm-diameter pots in May 2013. As  
150 roots were equally limited in depth for all the populations in pots, plant drought survival  
151 mirrored plants dehydration tolerance under a similar soil water availability (Volaire,  
152 2008). It allowed us to discount the effect of rooting depth associated mainly with  
153 dehydration avoidance (Pérez-Ramos et al., 2013, Volaire and Lelievre, 2001). For this  
154 experiment, the quantity of dry substrate was measured to be identical for each of the 4L  
155 pot so that the time dynamics of soil moisture could be accurately assessed and compared  
156 simply by pot weight during the period of intense water deficit in summer 2014.



157 - Experiment 2 was carried out to measure embolism threshold and stem anatomy.  
158 Individuals started from tillers taken from the adult plants sown in 2013 were transplanted  
159 in September 2014 into 10L (24 cm-diameter) pots in order to obtain reproductive tillers  
160 from spring 2015.

161 - Experiment 3 was carried out to measure biomechanical and leaf traits. Individuals taken  
162 from the initial adult plants were transplanted into new 4L pots in September 2015.

163 Each 4L and 10L pot contained four clones from a single population, to ensure soil cover close to  
164 field conditions, with 10 and 4 replicates for 4L pots and 10L pots respectively per population.

165 Pots were filled with a substrate composed of 65% local loamy-clay soil and 35% compost.

166 For all experiments, the plants were grown under an open-ended hoop-house and they were kept  
167 fully irrigated and fertilized (40 U N after each cut) except during the imposition of the severe  
168 drought in Experiment 1 during summer 2014. Pesticides were applied to prevent the  
169 development of diseases caused by insects (aphids) or fungi (rust). For all experiments, pots were  
170 distributed on a bench according to a full randomization design. In addition, the position of pots  
171 was modified multiple times during the experiment to minimize placement effects.

172 In addition, plants of one of the Mediterranean populations (Kasbah), that was grown in a nearby  
173 field in a 1.30 m deep loamy soil since 2011 (Barkaoui et al., 2016) and without irrigation nor  
174 fertilization for two years, were used for stem sampling in order to assess the plasticity of  
175 embolism resistance in one population. Twenty stems of this field-grown 'Kasbah' population  
176 were collected in spring 2015 and processed for the measurement of stem embolism (see below).  
177 No other measurement was carried out on these plants.

178 Mean temperatures were 9.5°C in winter, 16.6°C in spring and 24.2°C in summer, with 63%  
179 mean air relative humidity and mean radiation of  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  from spring to summer.

180

## 181 *Measurements*

182

### 183 *Dehydration tolerance under severe summer drought (Experiment 1)*

184 Plants of Experiment 1 were kept fully irrigated and fertilized until June 2014 when the severe  
185 summer drought started to be imposed as follows. In June 2014, the soil water content was raised  
186 to field capacity (60% soil water content for the substrate used) and irrigation was ceased  
187 thereafter. Pots were weighed at frequent intervals (every 2-5 days) throughout the experiment to

188 monitor the decrease in soil water content. Once full leaf senescence was reached for all  
189 populations, and when soil water content in the pots decreased to near 12% (27 to 35 days after  
190 irrigation cessation), plants were harvested at 3cm from the soil surface and the pot was put back  
191 under full irrigation (field capacity), thus ending the severe drought. All pots were re-watered  
192 after they reached the same soil water content to ensure that plant survival corresponded to an  
193 identical soil final dehydration for all populations irrespective of their growth potential. The  
194 plants were irrigated for 15 days after which dehydration survival was measured. Plants that did  
195 not produce new shoots after 15 days were counted as dead. The survival rate was measured as  
196 the number of living plants divided by the total number of plants (plant survival; SURV %).

197  
198 *Embolism Resistance* (Experiment 2)  
199 In late spring 2015, three pots for each population were maintained under irrigated conditions and  
200 allowed to flower. Stems were then collected and processed using the static centrifuge technique  
201 (Alder et al., 1997). A negative pressure was applied to stem pieces in a standard centrifuge with  
202 a custom-built, 26 cm rotor. Each stem segment was only spun once in the centrifuge. After  
203 centrifugation, a 2 cm long segment containing a node was excised under water from the middle  
204 of the stem segment. The segment was connected to the XYL'EM apparatus (Bronkhorst,  
205 Montigny-les-Cormeilles, France) to measure its hydraulic conductance using a solution of 10  
206 mM KCl and 1mM CaCl<sub>2</sub> in deionized ultrapure water. The sample was then resaturated with  
207 pressurised water to determine the percentage of loss of conductance caused by embolised  
208 conduits. For each pressure point in the vulnerability curve (VC), one to seven grass stems were  
209 used with 14 to 36 pieces per curve (min-max), and one S-shaped curve per population was fitted  
210 (Fig. S1) according to a sigmoid function (Pammenter and Vander Willigen, 1998). Embolism  
211 resistance was expressed by the 50% or 88% percentage loss of hydraulic conductance ( $P_{50}$ ,  $P_{88}$ ).  
212  $P_{50}$  is the metrics most used to compare hydraulic thresholds between species and populations  
213 while  $P_{88}$  was shown to be the embolism threshold leading to irreversible drought damage in  
214 angiosperm trees (Urli et al., 2013)

215  
216 *Stem anatomy*

217 To evaluate which stem characters contribute to the variation in embolism resistance observed  
218 among the *D. glomerata* populations, light microscopy observations were performed on cross

219 sections of stems – previously used for the measurement of embolism resistance – at the level of  
 220 internodes (from three stems per population). After vulnerability curve determination, samples  
 221 were frozen until processed for anatomy. Samples were defrost in water, stored in 50% ethanol  
 222 and then were embedded with LR White (hard grade, London Resin, UK) following (Hamann et  
 223 al., 2011), sectioned with a rotary microtome (Leica RM 2265) equipped with disposable blades.  
 224 Transverse sections of 4  $\mu\text{m}$  were made, heat-attached to the slides, stained with toluidine blue,  
 225 and mounted in Entellan®. The slides were observed with a Leica DM2500 light microscope  
 226 equipped with a Leica DFC-425C digital camera (Leica Microscopes, Wetzlar, Germany). A  
 227 range of stem anatomical characters (Lens et al., 2016), such as total stem area ( $A_S$ ), area of  
 228 lignified outer stem tissue ( $A_{LIG}$ ), surface area of fibre wall ( $A_{FW}$ ) and fibre lumen ( $A_{FL}$ ),  
 229 thickness of the metaxylem vessel wall ( $T_{VW}$ ), hydraulically weighted diameter of vessels ( $D_{HV}$ ),  
 230 and traits derived from these measurements, such as the proportion of lignified tissue per total  
 231 stem area ( $P_{LIG}$ ), the proportion of cell wall per fiber ( $P_{CWF}$ ), the proportion of total fiber wall  
 232 area in lignified area ( $P_{FWF} * A_{LIG}$ ), the proportion of total fiber wall in lignified area per stem  
 233 area ( $(P_{FWF} * A_{LIG}) : A_S$ ). Observations were made based on three individuals per population and at  
 234 least 30 measurements per feature using ImageJ v 1.43 (National Institutes of Health, Bethesda,  
 235 Maryland, USA).

236 The diameter of the vessels ( $D_V$ ) was calculated based on the lumen area that was considered to  
 237 be a circle following the equation:

238

$$239 \quad D_V = \sqrt{\frac{4A}{\pi}} \quad \text{Eqn (1)}$$

240

241 where  $D$  is the vessel diameter and  $A$  is the area of the vessel. The hydraulically weighted vessel  
 242 diameter ( $D_{HV}$ ; weights diameters of vessels according to their hydraulic conductance) was  
 243 calculated following the equation (Sperry et al., 1994):

244

$$245 \quad Dhv = \frac{\sum D^5}{\sum D^4} \quad \text{Eqn (2)}$$

246

247 where  $D$  is the vessel diameter as measured in eqn. (1).

248

249 *Leaf traits under full optimum conditions (Experiment 3)*

250 In grasses, leaf tissue density can be assessed both by leaf dry matter content (Cornelissen et al.,  
251 2003) **which is a reasonable indirect measure of dry matter concentration (Shipley and Vu, 2002)**  
252 and resistance of the leaf to mechanical fracture, that can be measured in a variety of ways (Ang  
253 et al., 2008, Aranwela et al., 1999, Onoda et al., 2011). For experiment 3, in early spring (2016),  
254 at the time before reproductive stage, one fully extended leaf was collected from one plant per pot  
255 (four replicates). Fracture toughness was measured with a portable mechanical measuring device  
256 (Instron® Inspec 2200) adapted for cutting with a single razor cut, following Ang et al (Ang et  
257 al., 2008). Leaf blades were placed on the cutting platform across a gap 2 mm wide permitting  
258 the passage of the diagonally angled blade in the middle of each leaf blade (single edged blade,  
259 "Personna GEM, 0.23 mm thickness, Cat.# 71962). Leaf toughness, the energy required to shear  
260 the leaf cross section in  $\text{J m}^{-2}$  was calculated after subtracting initial elastic loading and final  
261 elastic unloading, as well as friction energy from the force distance curves. As in Ang et al.  
262 (2008), the initial cut was followed by a second pass that measured just the friction force  
263 generated by the blade against the cut surfaces of the leaf. Leaves were cut with a razor angle of  
264  $20^\circ$  and at a crosshead speed of  $0.1 \text{ mm s}^{-1}$ . Leaf thickness and width were measured with a  
265 binocular at the central vein of the leaf and at the widest part of the leaf respectively. The leaf  
266 was then immediately placed into a tube with the cut end submerged in deionized water and  
267 stored in the dark at  $4^\circ \text{C}$  for 24 h. The fresh and dry leaf biomass (g) were weighed to obtain leaf  
268 dry matter content (LDMC; Leaf dry matter content is the oven-dry mass (mg) of a leaf divided  
269 by its water-saturated fresh mass (g), expressed in  $\text{mg g}^{-1}$ ) following (Perez-Harguindeguy et al.,  
270 2013). This trait is used as a proxy for leaf density which is a trait more difficult to measure  
271 (Shipley and Vu, 2002).

272

273 **Statistical analysis**

274 All statistical analyses were performed with Rstudio (version 0.98.501). The effect of  
275 biogeographical origin was tested with anova ('lmtest' package) followed with Tukey's post hoc  
276 tests. A principal component analysis (PCA; 'ade4' package) was performed on the twelve  
277 variables showing significant differences between origins, scaled and centered, to visualize their  
278 covariations. The four bioclimatic variables ([www.worldclim.org](http://www.worldclim.org)) chosen to characterize the  
279 drought and frost occurrence in the sites of origins of the populations (annual precipitations,

280 precipitations of the driest quarter, aridity index (Trabucco et al., 2008) and minimum  
281 temperature of the coldest months) were used as supplementary variables in the PCA. Pairwise  
282 correlations between all measured traits for each season were evaluated using Pearson's method  
283 ('Hmisc' package). We tested the relationships between key-variables using simple linear  
284 regressions.

285

286

## 287 **RESULTS**

288 Across the twelve populations of *D. glomerata* studied,  $P_{50}$  ranged from -3.06 to -6.36 MPa (Fig.  
289 1a), while  $P_{88}$  ranged from -5.06 to -11.6 MPa (Fig. 1b). The Mediterranean populations had  
290 significantly lower  $P_{50}$  (-5.8 and -10.6 MPa respectively) than the Temperate populations (-3.3  
291 and -6.1 MPa respectively), while the Northern populations showed intermediate embolism  
292 thresholds (Table 2). In addition,  $P_{50}$  of the Mediterranean population Kasbah significantly  
293 differed according to the growing environment (Fig. 2), either in pots ( $P_{50} = -6.05 \pm 0.31$ ) or in the  
294 field ( $P_{50} = -5.21 \pm 0.29$ ).

295  $P_{50}$  and  $P_{88}$  measured on potted irrigated plants were both highly and more correlated ( $r=0.88$  and  
296  $0.89$ ,  $P<0.001$ , Table 3) with the mean precipitation of the driest quarter of the year than with the  
297 annual precipitation of the origin site. Both embolism thresholds were, however, less strongly  
298 correlated with the aridity index of the site of origin ( $r=0.60$ ,  $P=0.039$ ). The mean aridity index  
299 was 1.20 for Northern populations (less arid) compared to 0.82 (more arid) for Temperate  
300 populations despite of the greater rainfall in Temperate areas. In addition, no correlation was  
301 detected between cavitation resistance and minimum temperatures of the coldest months of the  
302 sites studied.

303 Plant dehydration survival in pots was significantly greater (Table 2) for the Mediterranean  
304 populations (58%) than for the group of Northern and Temperate ones (7 to 13%).

305 Although leaf thickness did not discriminate the populations according to their origin, leaf width  
306 almost doubled with increasing latitude from on average 4.3 mm in Mediterranean populations to  
307 8 mm in Northern ones. Mediterranean populations also had a higher LDMC (20% greater) and a  
308 very significantly greater leaf fracture toughness (2.5 fold greater) than the Northern and  
309 Temperate populations.

310 The Mediterranean populations were distinguished from the others by having thinner stems with  
 311 a higher fibre wall proportion in lignified area per stem (Table 2). The traits for Northern and  
 312 Temperate populations were mostly not significantly different although the proportion of total  
 313 fibre wall area in lignified area was greater in Northern populations. The thickness of metaxylem  
 314 vessel wall highly discriminated all origins and spanned from 1.4, 1.2 and 1.1  $\mu\text{m}$  in  
 315 Mediterranean Northern and Temperate populations respectively (Table 2).

316  $P_{88}$  was highly and better correlated to dehydration survival ( $r=-0.79$ , Table 3, Fig. 4a) and leaf  
 317 traits such as fracture toughness ( $r=-0.78$ , Table 3, Fig. 4b) and LDMC ( $r=-0.82$ , Table 3, Fig.  
 318 3c), than  $P_{50}$ . A highly significant correlation was found between both embolism thresholds  
 319 (Table 3, Fig. 5, 6) and the thickness of metaxylem vessel wall in stems ( $T_{VW}$ :  $r=-0.92$  for  $P_{50}$ ).  
 320 Notably no other stem anatomical trait was correlated with either embolism thresholds. In  
 321 contrast, plant dehydration survival was correlated to the total stem area ( $A_S$ :  $r=-0.66$ ) and  
 322 therefore with the area of lignified outer stem tissue ( $A_{LIG}$ :  $r=-0.61$ ) as well as with the proportion  
 323 of fibre wall in lignified area over the entire stem area ( $(P_{FW} \cdot A_{LIG}) : A_S$   $r=0.59$ ).

324 Overall, the covariation between LDMC, leaf fracture toughness, embolism thresholds and  
 325 dehydration tolerance was very significant (Table 3). Unexpectedly, leaf toughness was not  
 326 correlated with any trait associated with stem lignification over the entire data set. However, a  
 327 PCA (Fig. 7a, b) clearly illustrates that high embolism resistance and dehydration survival  
 328 associated with small and more lignified lamina and lignified stems discriminate the group of  
 329 Mediterranean populations. In contrast, Temperate and Northern populations were characterised  
 330 by low dehydration survival and embolism resistance, bigger leaves and stems with thicker-  
 331 walled fibres especially for Northern populations.

332

### 333 **DISCUSSION**

334 This study is the first to explore intraspecific variability of embolism resistance, whole plant  
 335 dehydration tolerance and associated traits in an herbaceous grass species. Our results show a  
 336 huge range of embolism thresholds amongst the twelve *D. glomerata* populations studied,  
 337 reflecting its biogeographical distribution and corresponding with some key traits.

338 *Large intraspecific variability in cocksfoot*

339 If we compare the interspecific range for  $P_{50}$  (Lens et al., 2016) observed within 18 Poaceae  
340 species native to similar mesic and xeric environments (-3.2 to -7.5 MPa) with the intraspecific  
341 variability in *D. glomerata* found here (-3.06 to -6.36 MPa), the overlap is striking. Only *Stipa*  
342 *pennata*, a grass native to arid rangelands exhibited a lower  $P_{50}$  (-7.5 MPa) compared to the  
343 Mediterranean populations of *D. glomerata*. In addition, this intra-specific variation is much  
344 greater than that reported for a dicot herbaceous perennial *Solidago Canadensis* sampled from  
345 dry to humid sites and showing  $P_{50}$  ranging from -2.37 to -3.08 MPa (Nolf et al., 2014). The  
346 intraspecific range in  $P_{50}$  variation within cocksfoot matches with most of the woody species  
347 studied in angiosperms (-2 to -4 MPa) and gymnosperms (-4 to -8 MPa), although far from the  
348 extreme embolism threshold (-18.8 MPa) found so far in the gymnosperm *Callitris tuberculata*  
349 (Larter et al., 2015). This intraspecific variability is strikingly large in comparison with former  
350 results on woody species. For instance, across 17 populations of *Fagus sylvatica* originating from  
351 sites in a large European latitudinal gradient with annual precipitations spanning from 563 to  
352 1362 mm,  $P_{50}$  was relatively constant from -2.8 to -3.2 MPa (Wortemann et al., 2011). No  
353 significant differences in embolism resistance (-3.9 to -4.1 MPa) was found either across 24  
354 populations of *Pinus pinaster* from Northern African and French sites (Lamy et al., 2011).  
355 Nevertheless, plasticity in vulnerability of embolism (from -3 to -6 MPa) was shown in the very  
356 dry limits of *Pinus canariensis* under a strong aridity gradient (Lopez et al., 2016). **It could be**  
357 **interesting to explore whether** the high embolism resistance of *D. glomerata* from the most arid  
358 sites ( $P_{50} < -5$  MPa) could be ascribed to a shallower rooting system than woody species by  
359 analogy with shrubs that were found to be more embolism tolerant than trees in the same habitat,  
360 probably reflecting differences in rooting depth and drought tolerance (Brendel & Cochard,  
361 2011). The remarkable range in embolism resistance could be added to the list of factors  
362 contributing to explain why cocksfoot covers a greater span of biomes than that of most woody  
363 species.

364 Our intraspecific comparison is based on plants grown in similar optimum pot conditions for two  
365 years, underlining that the large differences within populations are genetically determined. **Yet,**  
366 **our results also show a high plasticity of embolism threshold since for the tested Kasbah**  
367 **population,  $P_{50}$  significantly varied between potted and field-grown plants of the same**  
368 **population. Many factors differ such as plant age at sampling date as well as many biotic and**  
369 **abiotic factors (soil depth, plant density...) between these two growing conditions for the same**

370 population. However, it may be hypothesized that a less negative  $P_{50}$  of field-growing plants may  
371 be ascribed in particular to a deeper rooting system (130 cm in the field versus 20 cm in pots)  
372 which enhances dehydration avoidance versus dehydration tolerance and may have impacted  
373 hydraulic adaptations (Scholz et al., 2012). It could be interesting to measure embolism resistance  
374 in the same populations of cocksfoot growing all in a similar deep soil in order to analyse the  
375 impact of the expression of full rooting depth on embolism thresholds. However, it can therefore  
376 be assumed that if sampling was done using populations growing in their natural environment,  
377 our results would have probably generated stronger genotype x environments interactions, and  
378 even further increased the range of intra-specific variability in embolism thresholds. Significant  
379 site x population interactions were for instance found in beech (Herbette et al., 2010, Wortemann  
380 et al., 2011) underlining the high phenotypic plasticity of embolism resistance. Further work is  
381 necessary to assess intra-specific  $P_{50}$  and  $P_{88}$  plasticity as a response to environments (e.g. soil  
382 depth, soil nutrients and water availability).

### 383 *Relationships between embolism resistance and environmental limiting factors*

384 Our results show a correlation between the annual and driest quarter precipitations of the sites  
385 studied with embolism thresholds measured on plants grown in a common garden under optimum  
386 irrigation. As a general pattern, dehydration survival in pots was greater for the populations  
387 coming from the driest sites with  $P_{50}$  and  $P_{88}$  more negative than -4.50 and -9.3 MPa respectively.  
388 Plant mortality under severe drought when the resource is limited (shallow soils) can be then  
389 predicted by the hydraulic failure model assuming that a significant loss of conductivity can  
390 trigger plant mortality (McDowell et al., 2013). Our results are in line with a meta-analysis of tree  
391 species showing that hydraulic traits capture key mechanisms determining tree death (Anderegg  
392 et al., 2016). However, embolism resistance was less strongly correlated with the aridity index of  
393 the site of origin in contradiction to a former study dealing with a range of Temperate to semi-  
394 arid grass species that showed a strong correlation between  $P_{50}$  and aridity (Lens et al., 2016). It  
395 would be interesting to test whether this interspecific correlation would hold when including  
396 herbaceous species from the highest latitudes. In *D. glomerata*, Northern populations showed a  
397 trend to more negative  $P_{50}$  values than Temperate populations and are more frost tolerant,  
398 suggesting that the Northern populations could have also developed greater embolism resistance  
399 as a mechanism of frost tolerance (Lens et al., 2013). Both drought and freezing-thawing of stems



400 induce a loss of hydraulic conductivity in woody plants (Feng et al., 2015). Frost ‘drought’ had a  
401 large effect on plant water transport, adaptations in hydraulic safety and related anatomical  
402 parameters (Mayr et al., 2006) and the propensity for freeze induced embolism increases as  
403 conduit diameter increases (Zanne et al., 2014). The relationships between recovery from winter  
404 embolism and anatomical vessel traits also varied according to functional groups of tree species  
405 (Niu et al., 2017). The role of hydraulic dysfunction under frost remains to be explored in  
406 herbaceous species.

#### 407 *Tissue lignification and embolism resistance*

408 In pseudostems, partly constituted with leaf sheaths, we found a strong positive correlation  
409 between the thickness of the metaxylem vessel wall and embolism resistance while lignification  
410 characters are not correlated with  $P_{50}$  nor  $P_{88}$ . Despite a significant positive correlation (Lens et  
411 al., 2016) between embolism resistance and stem lignification amongst 19 grass species (in terms  
412 of the thickness of the fibre walls and the relative proportion of lignified tissue per stem area),  
413 this trade-off is lacking within populations of *D. glomerata*. This lack of correlation is  
414 noteworthy given the huge range in  $P_{50}$  variation within cocksfoot. There are slight indications  
415 that stem lignification may contribute to differences in embolism resistance. For instance, Figure  
416 6ab shows that the proportion of lignified area per stem area clusters with other key traits for the  
417 embolism resistant Mediterranean populations. Furthermore, the two most resistant  
418 Mediterranean populations have more lignified fibres inside and surrounding the vascular bundles  
419 (Fig. S2) compared to the other cocksfoot populations studied. However, this character is difficult  
420 to quantify and seemingly shows at random variation amongst the remaining populations. Since  
421 we have sectioned internodes, we had to restrict ourselves to measurements of solitary vessels. It  
422 is plausible that our observations also reflect a correlation between more negative  $P_{50}/P_{88}$  values  
423 and increased inter-vessel wall thickness compared to vessel diameter (so called thickness-to-  
424 span ratio) in case of vessel multiples that are much more present in the nodal parts of the stem.  
425 Vessel wall reinforcement was suggested to be required for embolism resistance in order to  
426 prevent wall implosion when xylem pressure is very negative (Hacke et al., 2001). However,  
427 vessels have never been observed to implode in stems, probably because embolism events occur  
428 before the critical vessel implosion threshold is reached. Whatever the exact mechanism(s)  
429 operating during xylem embolism dysfunction in *D. glomerata*, it appears that plants with more

430 fracture resistant leaves have stems that are more resistant to embolism than plants with less  
431 resistant leaves. In addition to possibly reducing the probability of lethal embolism (Hacke and  
432 Sperry, 2001), mechanically robust leaf architecture will also play a role in mechanically  
433 supporting water-stressed leaves and protecting leaf meristems as discussed below. This perhaps  
434 at least partly explains the tendency towards narrower, more compact, denser, tougher leaves in  
435 the Mediterranean ecotypes that are more likely exposed to more drought conditions. Of course  
436 leaf lamina traits are anatomically and functionally grade into the more proximal leaf sheath  
437 (Bell, 1991) which is also important for mechanical robustness and resistance to dehydration of  
438 the self-supporting meristems of *D. glomerata*.

439 *Drought survival in perennial herbaceous species: hydraulic leaf traits may not be crucial*

440 A general tenet of ecophysiology of drought adaptation is based on the assumption of a strong  
441 linkage between hydraulic and stomatal traits especially in woody species (Skelton et al., 2015,  
442 Bartlett et al., 2016) and also in herbaceous species (Holloway-Phillips and Brodribb, 2011, Lens  
443 et al., 2016, Nardini et al., 2012). Regarding the extreme case of drought plant mortality in both  
444 annual and perennial grasses, basal leaf meristems are the tissues surviving longer and able to  
445 regenerate when the mature blades are dead (Van Peer et al., 2004). These leaf meristems  
446 enclosed in mature leaf sheaths, are protected from intense evaporation and can survive more  
447 intense water deficits than older tissues (Barlow et al., 1980, Munns et al., 1979). As they  
448 accumulate carbohydrates such as fructans (Schnyder and Nelson, 1989, Volaire et al., 1998),  
449 apices exhibit the greatest osmotic adjustment relative to other tissues during drought (Matsuda  
450 and Riazi, 1981, West et al., 1990). In addition, the strategy of summer dormancy (Gillepsie and  
451 Volaire, 2017, Volaire and Norton, 2006) found in some species and populations of perennial  
452 species, triggers leaf senescence and meristem dehydration to reduce plant metabolism and  
453 consequently enhance drought survival. Our results show that the Mediterranean populations of  
454 *D. glomerata* which are the most summer dormant, ‘sacrifice’ their lamina in summer  
455 (irrespective of the water supply) but also have the highest embolism resistance and  
456 dehydration tolerance. In frost-prone areas too, perennial herbaceous species avoid freezing by  
457 senescing aboveground tissues and overwintering as underground storage organs (Zanne et al.,  
458 2014). For dehydration tolerance under frost or drought (Gillepsie and Volaire, 2017), these  
459 model species can then raise the relevance of the paradigm of hydraulic safety margins in leaves

460 for stress survival. The leaf (lamina)-level drought tolerance (Nardini and Luglio, 2014,  
461 Ocheltree et al., 2016) may not be the key-level to explore drought survival in all species.  
462 However, studies linking hydraulic conductivity and lamina anatomy (Scoffoni et al., 2017) could  
463 be fruitful provided they take into account the continuum between expanded blades, sheaths and  
464 meristematic tissues. The co-variation between hydraulic traits and anatomical traits in meristems  
465 but also in root tissues should be explored further in grasses.

466 In conclusion, this study investigated dehydration survival amongst twelve populations of *D.*  
467 *glomerata* from a latitudinal gradient from Mediterranean to Scandinavian areas, representing  
468 only a restricted range of its much larger biogeographical distribution. *D. glomerata* represents an  
469 ‘ideal’ species for analyzing the interactions genotype x environment because of its  
470 biogeographical distribution is huge and spans over Europe and Asia (Borrill, 1991) and its  
471 phylogenetic relationships between populations are being investigated and show high genetic  
472 variation within both populations and geographical origins (Lumaret, 1988, Xie et al., 2010).  
473 Compared to woody species, the large degree of intra-specific variability in dehydration tolerance  
474 and embolism resistance within cocksfoot has consequences for its sensitivity to climate shifts,  
475 possible migrations and future biogeographical distribution (Anderegg, 2015). In addition, given  
476 that the measurement of plant drought survival is experimentally difficult to standardize, our  
477 study demonstrating the key role of embolism threshold and tissue lignification as proxy for  
478 dehydration tolerance provide highly relevant knowledge to screen genetic resources and new  
479 breeding material.

480 Finally, this study calls for extending the numerous studies on mortality factors and lethal  
481 embolism thresholds in woody species to a large range of herbaceous species, both at inter and  
482 intra-specific levels, under contrasting environmental constraints.

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752

Population	Origin	Country	Latitude	Longitude	Temperature (°C)			Rainfall mean (mm)			Aridity Index
					Annual mean	Min of the coldest month	Max of the warmest month	Annual	Wettest quarter	Driest quarter	
Maroc 1(ma1)	Mediterranean	Morocco	33.10	-8.08	18.0	7.0	30.2	346	7	4	0.27
Maroc 9 (ma9)	Mediterranean	Morocco	29.71	-8.96	16.2	1.9	31.1	246	13	5	0.17
Kasbah (cv kas)	Mediterranean	Morocco	32.35	-6.16	11.7	3.9	39.6	270	215	17	0.31
Sicily (sic)	Mediterranean	Italy	37.48	14.50	15.4	4.0	27.6	572	237	40	0.47
Carnac (car)	Temperate	France	47.58	-3.07	11.7	3.5	21.6	893	179	153	0.86
Fourchette (fou)	Temperate	France	48.17	-2.75	10.8	2.3	20.8	857	174	153	0.74
St Michel (stm)	Temperate	France	48.63	-1.51	11.5	2.6	22.3	731	169	149	0.78
Ludac (cv lud)	Temperate	France	46.58	0.33	11.5	9	25.0	724	152	152	0.90
Danemark (dan)	Northern	Denmark	56.46	8.66	7.8	-3.2	19.4	809	201	134	1.48
Sweden (swe)	Northern	Sweden	60.50	24.22	4.8	-9.8	21.4	613	199	93	1.10
Loke (cv lok)	Northern	Sweden	62.98	14.80	2.6	-12.6	18.9	563	213	87	1.14
Tammisto (cv tam)	Northern	Finland	60.23	23.75	4.9	-9.3	21.3	609	196	92	1.08

753

754 **Table 1.** List of the twelve populations of *Dactylis glomerata*. Climatic data associated either with their origin sites of collection or  
755 sites of breeding (for cultivars cv in Temperate and Northern areas) from the WorldClim data set (<http://www.worldclim.org>; (Hijmans  
756 et al., 2005). Seeds from Northern populations were provided by the Nordic Genetic Resource Center (NordGen). Temperate  
757 populations were provided by the plant genetic resources information system of the National Institute for Agronomical Research  
758 (INRA, France, Lusignan). The Sicilian ecotype came from University of Catania, Italy. The Moroccan ecotypes came from INRA  
759 Rabat, Morocco

760 **Table 2:** Results of variance analysis and Tukey test between means of variables for the three  
 761 origins of *Dactylis glomerata* populations. \*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; ns: non-  
 762 significant. For each variable, different subscript letters indicate significant differences between  
 763 origins  
 764

	Biogeographic origin of <i>D. glomerata</i> populations			
	P value	Medit.	Northern	Temperate
SURV = Plant dehydration survival (%)	<0.001***	58.2 <sup>a</sup>	13.1 <sup>b</sup>	6.9 <sup>b</sup>
P <sub>50</sub> = Embolism pressure threshold for 50% embolism (MPa)	0.0016**	-5.8 <sup>a</sup>	-4.3 <sup>ab</sup>	-3.3 <sup>b</sup>
P <sub>88</sub> = Embolism pressure threshold for 88% embolism (MPa)	<0.001***	-10.4 <sup>a</sup>	-7.6 <sup>ab</sup>	-6.1 <sup>b</sup>
Lwidth = Leaf width (mm)	<0.001***	4.28 <sup>a</sup>	7.97 <sup>c</sup>	6.22 <sup>b</sup>
Lthick = Leaf thickness (mm)	0.13ns	0.30	0.36	0.35
LDMC = Leaf Dry Matter content (mg g DM <sup>-1</sup> )	<0.001***	232.72 <sup>a</sup>	185.75 <sup>b</sup>	188.41 <sup>b</sup>
LFrac = Leaf fracture resistance (J m <sup>-2</sup> )	<0.001***	478.6 <sup>a</sup>	188.47 <sup>b</sup>	225.55 <sup>b</sup>
A <sub>S</sub> = Total stem area (mm <sup>2</sup> )	0.006**	2.07 <sup>a</sup>	3.09 <sup>b</sup>	2.9 <sup>b</sup>
A <sub>LIG</sub> = lignified area (μm <sup>2</sup> )	0.02*	0.27 <sup>a</sup>	0.38 <sup>b</sup>	0.33 <sup>ab</sup>
P <sub>LIG</sub> = Proportion of lignified area per total stem area	0.02*	0.1308 <sup>b</sup>	0.1258 <sup>ab</sup>	0.115 <sup>a</sup>
A <sub>F</sub> = fiber cell area (μm <sup>2</sup> )	0.34ns	70.61	77.21	77.56
A <sub>FL</sub> = fiber lumen area (μm <sup>2</sup> )	0.50ns	15.95	14.16	14.76
A <sub>FW</sub> = fiber wall area (μm <sup>2</sup> )	0.12ns	54.66	63.04	62.79
P <sub>CWF</sub> = Proportion of cell wall per fiber	0.029*	0.77 <sup>a</sup>	0.82 <sup>b</sup>	0.81 <sup>ab</sup>
P <sub>FWF</sub> *A <sub>LIG</sub> = Proportion of total fiber wall area in lignified area	0.037*	0.30 <sup>ab</sup>	0.33 <sup>b</sup>	0.29 <sup>a</sup>
(P <sub>FWF</sub> *A <sub>LIG</sub> ):A <sub>S</sub> = Proportion of total fiber wall in lignified area per stem area	0.002**	0.156 <sup>a</sup>	0.117 <sup>b</sup>	0.102 <sup>b</sup>
D <sub>HV</sub> = hydraulically weighted diameter of vessels (μm)	0.17ns	23.04	21.77	21.18
T <sub>VW</sub> = Thickness of metaxylem vessel wall (μm)	<0.001***	1.426 <sup>c</sup>	1.226 <sup>b</sup>	1.109 <sup>a</sup>

**Table 3:** Pearson coefficients of correlations (lower part of the table) and P values (top part of the table) between traits measured on twelve populations of *Dactylis glomerata*. For abbreviations and units see table 1 and 2. Climatic variables of the origin sites (or breeding sites) of the populations: P<sub>DRY</sub>= Mean precipitations of the driest three months; P<sub>YEAR</sub> = Mean annual precipitations, T<sub>MIN</sub> = mean minimum temperatures of the coldest months, Aridity = aridity index. P<0.05 (italics, bold), P<0.01 (bold), P<0.001 (bold underlined)

	P <sub>88</sub>	P <sub>50</sub>	SURV	Lwidth	Lthick	LDMC	LFrac	A <sub>S</sub>	A <sub>LIG</sub>	P <sub>LIG</sub>	A <sub>F</sub>	A <sub>FL</sub>	A <sub>FW</sub>	P <sub>CWF</sub>	P <sub>FWF*</sub> A <sub>LIG</sub>	(P <sub>FWF*</sub> A <sub>LIG</sub> ) :A <sub>S</sub>	D <sub>HV</sub>	T <sub>VW</sub>	P <sub>SUM</sub>	P <sub>YEAR</sub>	T <sub>MIN</sub>	Aridity
P <sub>88</sub>	1	0,00	0,00	0,10	0,19	0,00	0,00	0,13	0,18	0,89	0,45	0,67	0,38	0,37	0,88	0,14	0,09	0,00	0,00	0,00	0,76	0,04
P <sub>50</sub>	<b><u>0,94</u></b>	1	0,02	0,21	0,25	0,02	0,03	0,24	0,45	0,58	0,51	0,47	0,38	0,27	0,73	0,18	0,33	0,00	0,00	0,00	0,92	0,04
SURV	<b><u>-0,79</u></b>	<b><u>-0,65</u></b>	1	0,01	0,02	0,00	0,00	0,02	0,03	0,85	0,09	0,73	0,06	0,13	0,80	0,04	0,07	0,11	0,00	0,01	0,25	0,01
Lwidth	0,50	0,39	<b><u>-0,71</u></b>	1	0,01	0,00	0,00	0,00	0,02	0,40	0,14	0,87	0,16	0,35	0,15	0,09	0,71	0,36	0,10	0,21	0,00	0,00
Lthick	0,40	0,36	<b><u>-0,66</u></b>	<b><u>0,72</u></b>	1	0,02	0,02	0,20	0,42	0,53	0,08	0,48	0,04	0,07	0,30	0,73	0,81	0,78	0,06	0,12	0,03	0,01
LDMC	<b><u>-0,82</u></b>	<b><u>-0,68</u></b>	<b><u>0,87</u></b>	<b><u>-0,85</u></b>	<b><u>-0,65</u></b>	1	0,00	0,01	0,07	0,50	0,23	0,66	0,18	0,22	0,64	0,07	0,13	0,07	0,00	0,04	0,09	0,01
LFrac	<b><u>-0,78</u></b>	<b><u>-0,61</u></b>	<b><u>0,92</u></b>	<b><u>-0,84</u></b>	<b><u>-0,68</u></b>	<b><u>0,97</u></b>	1	0,02	0,03	0,81	0,21	0,62	0,16	0,18	0,62	0,08	0,10	0,11	0,01	0,05	0,08	0,00
A <sub>S</sub>	0,46	0,37	<b><u>-0,66</u></b>	<b><u>0,83</u></b>	0,40	<b><u>-0,70</u></b>	<b><u>-0,67</u></b>	1	0,00	0,24	0,15	0,22	0,30	0,99	0,14	0,00	0,90	0,30	0,11	0,19	0,08	0,13
A <sub>LIG</sub>	0,41	0,24	<b><u>-0,61</u></b>	<b><u>0,65</u></b>	0,26	-0,54	<b><u>-0,62</u></b>	<b><u>0,78</u></b>	1	0,36	0,24	0,24	0,42	0,88	0,06	0,08	0,71	0,34	0,30	0,49	0,17	0,13
P <sub>LIG</sub>	-0,04	-0,18	0,06	-0,27	-0,20	0,22	0,08	-0,37	0,29	1	0,81	0,80	0,87	0,96	0,67	0,18	0,37	0,92	0,53	0,42	0,52	0,99
A <sub>F</sub>	0,24	0,21	-0,52	0,45	0,53	-0,37	-0,39	0,44	0,37	-0,08	1	0,66	0,00	0,06	0,09	0,99	0,87	0,96	0,19	0,23	0,41	0,32
A <sub>FL</sub>	-0,14	-0,23	0,11	0,05	-0,22	0,14	0,16	0,38	0,37	-0,08	0,14	1	0,64	0,01	0,05	0,76	0,21	0,64	0,26	0,17	0,92	0,10
A <sub>FW</sub>	0,28	0,28	-0,55	0,43	<b><u>0,59</u></b>	-0,41	-0,44	0,33	0,26	-0,05	<b><u>0,96</u></b>	-0,15	1	0,00	0,28	0,92	0,60	0,93	0,09	0,10	0,43	0,13
P <sub>CWF</sub>	0,28	0,35	-0,46	0,29	0,53	-0,38	-0,41	0,00	-0,05	-0,02	0,56	<b><u>-0,73</u></b>	<b><u>0,77</u></b>	1	0,60	0,90	0,21	0,65	0,06	0,03	0,49	0,02
P <sub>FWF*</sub> A <sub>LIG</sub>	-0,05	-0,11	-0,08	0,44	0,33	-0,15	-0,16	0,45	0,56	0,14	0,52	<b><u>0,59</u></b>	0,34	-0,17	1	0,60	0,11	0,68	0,69	0,53	0,20	0,78
(P <sub>FWF*</sub> A <sub>LIG</sub> ):A <sub>S</sub>	-0,45	-0,42	<b><u>0,59</u></b>	-0,50	-0,11	0,53	0,53	<b><u>-0,76</u></b>	-0,52	0,41	0,00	-0,10	0,03	0,04	0,17	1	0,59	0,16	0,08	0,08	0,47	0,24
D <sub>HV</sub>	-0,51	-0,31	0,53	-0,12	-0,08	0,46	0,50	0,04	-0,12	-0,28	-0,05	0,39	-0,17	-0,39	0,49	0,17	1	0,44	0,19	0,37	0,97	0,36
T <sub>VW</sub>	<b><u>-0,85</u></b>	<b><u>-0,92</u></b>	0,48	-0,29	-0,09	0,53	0,49	-0,33	-0,30	0,03	0,02	0,15	-0,03	-0,14	0,13	0,44	0,25	1	0,01	0,01	0,96	0,09
P <sub>SUM</sub>	<b><u>0,88</u></b>	<b><u>0,89</u></b>	<b><u>-0,84</u></b>	0,50	0,55	<b><u>-0,77</u></b>	<b><u>-0,74</u></b>	0,48	0,32	-0,20	0,40	-0,35	0,51	0,56	-0,13	-0,53	-0,40	<b><u>-0,71</u></b>	1	0,00	0,83	0,01
P <sub>YEAR</sub>	<b><u>0,75</u></b>	<b><u>0,86</u></b>	<b><u>-0,71</u></b>	0,39	0,48	<b><u>-0,61</u></b>	-0,57	0,40	0,22	-0,26	0,38	-0,42	0,50	<b><u>0,61</u></b>	-0,20	-0,52	-0,29	<b><u>-0,70</u></b>	<b><u>0,94</u></b>	1	0,83	0,01
T <sub>MIN</sub>	0,10	-0,03	0,36	<b><u>-0,82</u></b>	<b><u>-0,62</u></b>	0,51	0,53	-0,53	-0,42	0,21	-0,26	-0,03	-0,25	-0,22	-0,40	0,23	0,01	-0,02	-0,07	-0,07	1	0,03
Aridity	<b><u>0,60</u></b>	<b><u>0,60</u></b>	<b><u>-0,71</u></b>	<b><u>0,76</u></b>	<b><u>0,73</u></b>	<b><u>-0,74</u></b>	<b><u>-0,77</u></b>	0,47	0,46	0,01	0,32	-0,49	0,46	<b><u>0,65</u></b>	0,09	-0,37	-0,29	-0,51	<b><u>0,70</u></b>	<b><u>0,70</u></b>	<b><u>-0,62</u></b>	1

769 **Legend of figures**

770 **Fig. 1. Thresholds of embolism resistance in cocksfoot.** Means and standard errors of water  
 771 potentials at 50 % embolism (a,  $P_{50}$ ) and at 88% embolism (b,  $P_{88}$ ) in stems of twelve populations  
 772 of *Dactylis glomerata* of contrasting origins (red = Mediterranean; blue = Northern; green =  
 773 Temperate)

774

775 **Fig. 2. Embolism resistance at increasing water potentials in stems of the Mediterranean**  
 776 *Dactylis glomerata* population ‘Kasbah’ growing either in pot or in the field. Mean water  
 777 potentials at 50 % embolism ( $P_{50}$ ) are -6.1 MPa and -5.2 MPa for the population growing in pot  
 778 and in the field respectively.

779 **Fig. 3. Embolism resistance and aridity of origin site in cocksfoot.** Linear relationships  
 780 between mean precipitation of the driest quarter at origin site and water potential at 50%  
 781 embolism ( $P_{50}$ ) in twelve populations of *Dactylis glomerata* of contrasting origins (red =  
 782 Mediterranean; blue = Northern; green = Temperate). Bars correspond to standard errors.

783 **Fig. 4. Embolism resistance and (a) dehydration tolerance, (b) leaf fracture toughness and**  
 784 **(c) leaf dry matter content in cocksfoot.** Linear relationships between water potential at 88%  
 785 embolism ( $P_{88}$ ) and (a) the proportion of surviving plants at soil wilting point in short pots  
 786 (dehydration tolerance), (b) leaf fracture toughness of irrigated plants and (c) leaf dry matter  
 787 content (LDMC) of irrigated plants in twelve populations of *Dactylis glomerata* of contrasting  
 788 origins (red = Mediterranean; blue = Northern; green = Temperate). Bars correspond to standard  
 789 errors.

790

791 **Fig. 5 Embolism resistance and stem anatomy in cocksfoot.** Linear relationships between  
 792 water potential at 50% embolism ( $P_{50}$ ) with thickness of metaxylem vessel walls in stem in  
 793 twelve populations of *Dactylis glomerata* of contrasting origins (red= Mediterranean; blue=  
 794 Northern; green= Temperate). Bars correspond to standard errors.

795 **Fig. 6 Thickness of metaxylem vessel wall in stems of cocksfoot:** three populations of *Dactylis*  
 796 *glomerata* of contrasting origins: a = 'car' (Temperate); b = 'swe' (Northern); c = 'ma1'  
 797 (Mediterranean). **The arrows point to wall of the metaxylem vessels.**

798 **Fig. 7 PCA of 12 plant traits and 12 populations of cocksfoot.** Principal component analysis  
 799 (factorial axes 1 and 2) showing (a) the distribution of the twelve traits significantly  
 800 discriminating origins of populations (only P<sub>50</sub> was included since it is highly correlated to P<sub>88</sub>),  
 801 see table 2 and (b) twelve populations of *Dactylis glomerata* of contrasting origins (red=  
 802 Mediterranean; blue= Northern; green= Temperate). **Climatic data of the origin or breeding site:**  
 803 **Annual precipitations (P<sub>year</sub>), precipitations of the driest quarter (P<sub>dry</sub>), aridity index (aridity)**  
 804 **and minimum temperature of the coldest months (T<sub>min</sub>) were included as supplementary**  
 805 **variables (bold and italics characters).**

806

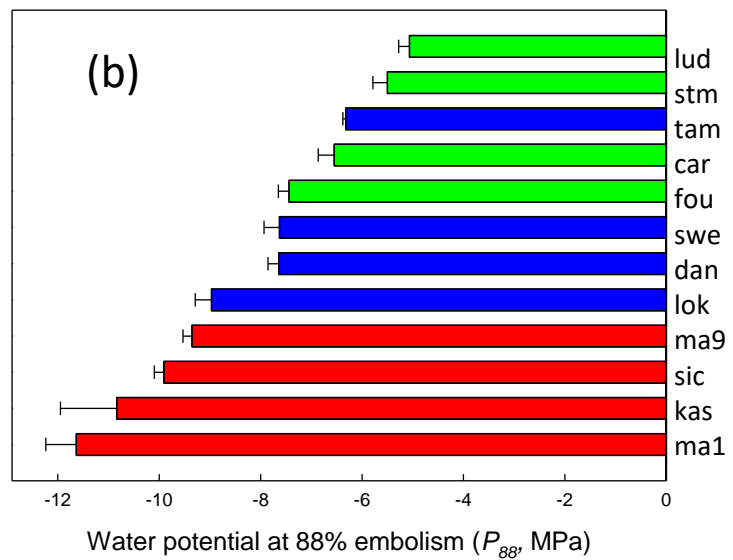
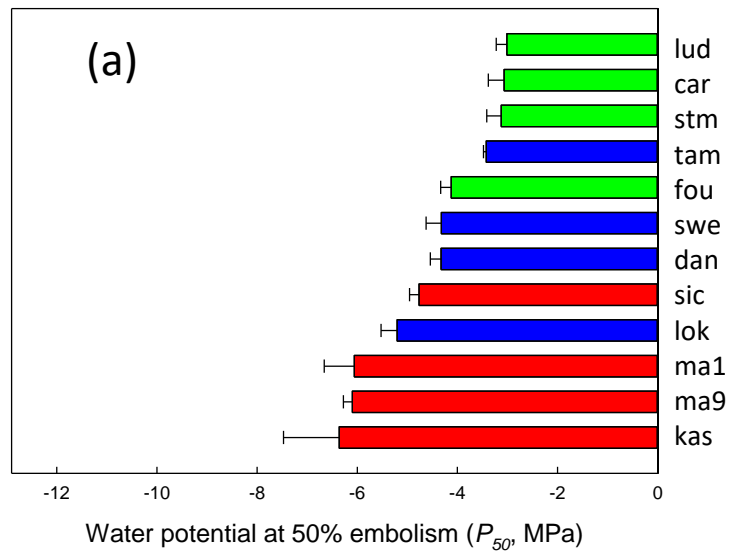
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## 809 **Supplementary material**

810 **S1: S curves for determination of embolism resistance in cocksfoot.** Relationships between  
 811 percentage of embolism and water potential measured for 12 populations of *Dactylis glomerata*  
 812 originating from Temperate (green), Northern (blue) and Mediterranean (red) areas. Fitted curves  
 813 are sigmoid functions. Vertical bars correspond to standard error, n = 1 to 7. P<sub>50</sub> and R<sup>2</sup> are given  
 814 for each curve

815 **S2: Overview of the stem anatomy of the 12 populations of *Dactylis glomerata* and P<sub>88</sub> (MPa)**





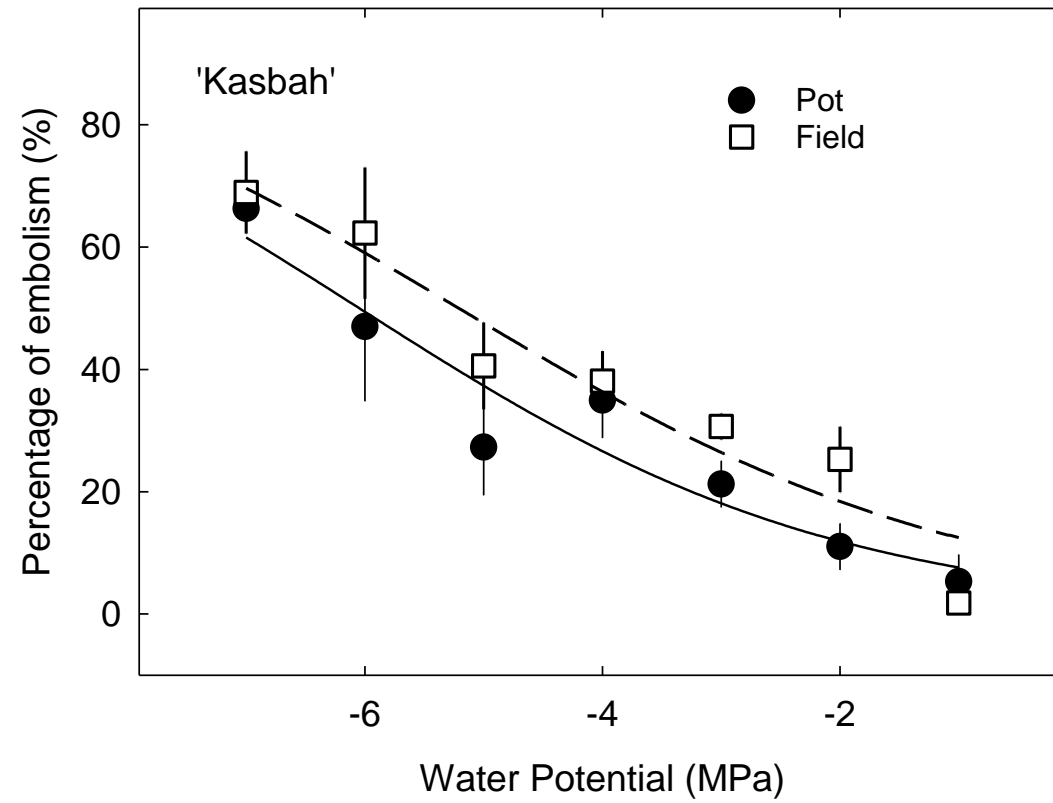


Fig.3

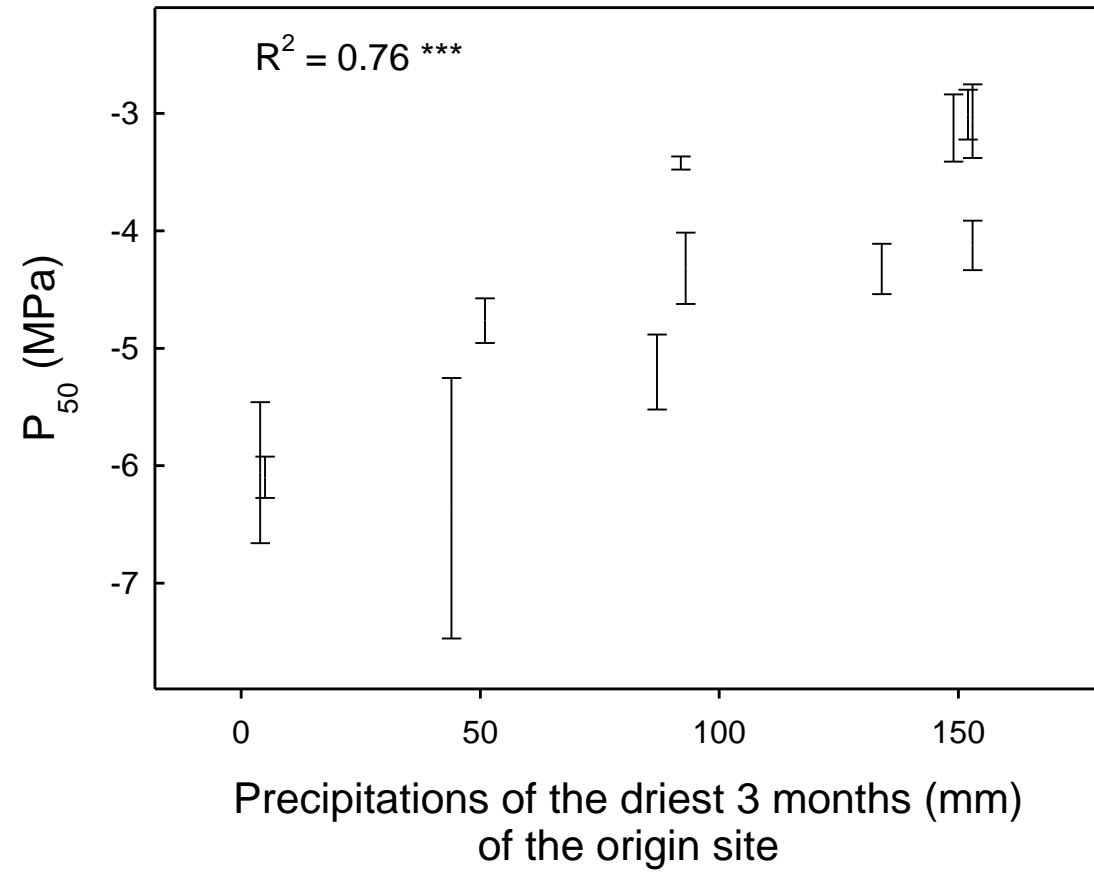


Fig. 4

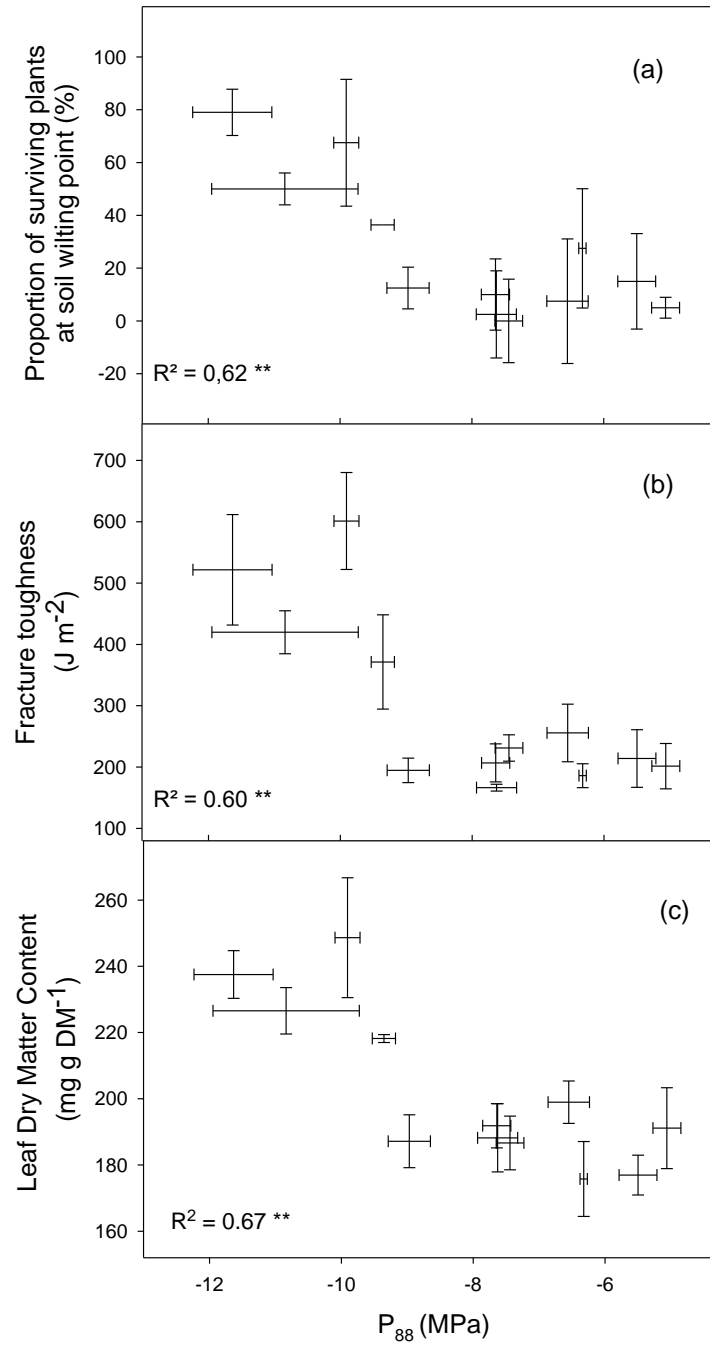


Fig. 5

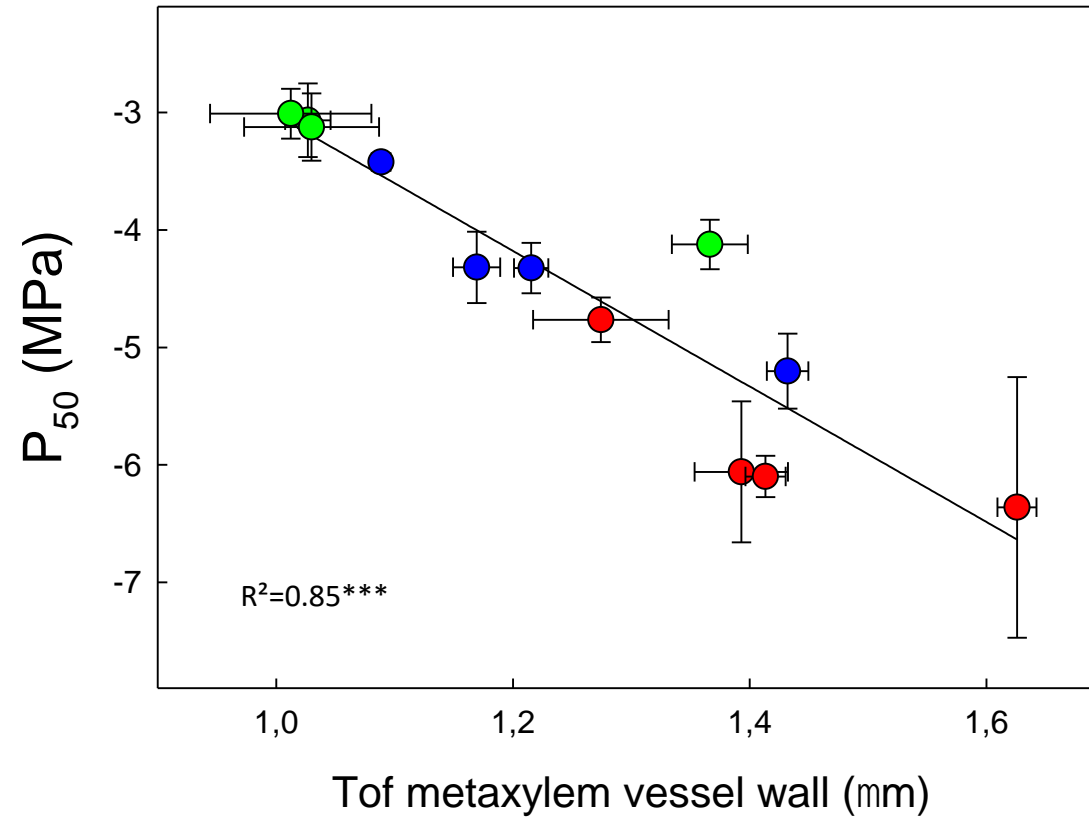


Fig. 6

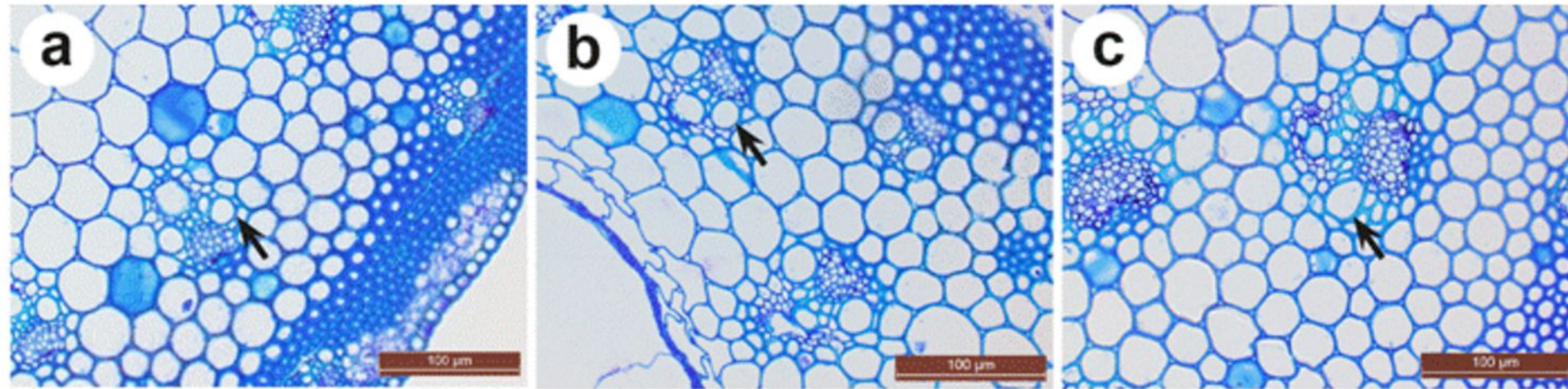


Fig. 7

