

1 **Short title:**  $\delta^2\text{H}$  reflects plant performance

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5 **Title:** The hydrogen isotope composition  $\delta^2\text{H}$  reflects plant performance

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19 **One-sentence summary:** The hydrogen isotope composition ( $\delta^2\text{H}$ ) exhibits specific  
20 features that report the water conditions of a wheat crop as well as the photosynthetic  
21 characteristics of the plant part considered.

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23 **Author contributions:** R.S.B. and J.L.A. conceived and designed the study; R.S.B.,  
24 J.B. and M.D.S. carried out the field measurements; R.S.B. and R.M.M. conducted  
25 laboratory work, R.S.B. and J.L.A. analyzed the data; R.S.B. and J.L.A. interpreted the  
26 results; R.S.B. took the principal role in writing the manuscript. All authors have  
27 contributed to the revision of the manuscript.

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39 **Summary**

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41 The stable carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotope compositions in plant matter reflect  
42 photosynthetic and transpirative conditions in plants, respectively. However, the nature  
43 of hydrogen isotope composition ( $\delta^2\text{H}$ ) and what it reflects of plant performance is  
44 poorly understood. Using durum wheat (*Triticum turgidum* var. *durum*), this study  
45 evaluated the effect of different water and nitrogen growing field conditions on  
46 transpiration and how this influenced the performance of  $\delta^2\text{H}$  in autotrophic (flag leaf),  
47 mixotrophic (ears) and heterotrophic (grains and roots) organs. Moreover,  $\delta^2\text{H}$  was  
48 compared to the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in the same organs. Isotope compositions were analyzed  
49 in dry matter, the water-soluble fraction, and in water from different tissues of a set of  
50 genotypes. Similar to  $\delta^{13}\text{C}$ , the  $\delta^2\text{H}$  correlated negatively with stomatal conductance,  
51 whereas no correlation was observed for  $\delta^{18}\text{O}$ . Moreover,  $\delta^2\text{H}$  was not only affected by  
52 changes in transpiration but also by photosynthetic reactions, probably as a consequence  
53 of NADPH formation in autotrophic organs. Compared to the  $\delta^2\text{H}$  of stem water, plant  
54  $\delta^2\text{H}$  was strongly diminished in photosynthetic organs like the flag leaves, whereas it  
55 strongly increased in heterotrophic organs such as grains and roots. In heterotrophic  
56 organs,  $\delta^2\text{H}$  was associated with post-photosynthetic effects as there are several  
57 processes that lead to  $^2\text{H}$ -enrichment of carbohydrates. In summary,  $\delta^2\text{H}$  exhibited  
58 specific features that inform about the water conditions of the wheat crop, together with  
59 the photosynthetic characteristics of the plant part considered. Moreover, correlations of  
60  $\delta^2\text{H}$  with grain yield illustrate that this isotope can be used to assess plant performance  
61 under different growing conditions.

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64

65 **INTRODUCTION**

66

67 Analyses of the stable isotope ratios of carbon and oxygen in plant material have been  
68 applied in time-integrated approaches for climatological, ecological or biochemical  
69 research in plant science (Dawson et al., 2002; Barbour, 2007; Gessler et al., 2014),  
70 including the evaluation of crop performance under different environmental conditions  
71 (Richards, 1996; Farquhar et al., 1998; Barbour and Farquhar, 2000; Araus et al. 2003,  
72 2013; Farquhar et al., 2007; Cabrera-Bosquet et al., 2009a, 2011). The stable isotope  
73 ratio of hydrogen in plant material has also been examined in different areas of plant  
74 research (Dawson et al., 2002). However, it has not been exploited in crop research to  
75 the same degree.

76

77 The carbon isotope composition ( $\delta^{13}\text{C}$ ) of plant dry matter, frequently expressed as a  
78 discrimination from surrounding air ( $\Delta^{13}\text{C}$ ), has been used for decades as a tool for  
79 screening plants with high water use efficiency during the assimilate deposition period  
80 due to the well-established link between  $\Delta^{13}\text{C}$  and the intercellular versus the  
81 atmospheric partial pressure of  $\text{CO}_2$  (Farquhar and Richards, 1984; Richards et al.,  
82 2002; Farquhar et al. 1989). In  $\text{C}_3$  plants,  $^{13}\text{C}$  discrimination mainly occurs during two  
83 steps of  $\text{CO}_2$  uptake: (i)  $\text{CO}_2$  diffusion from the air to the intercellular air space through  
84 the boundary layer and stomata and (ii) the carboxylation reaction by Rubisco (Farquhar  
85 et al., 1982). In addition, the water regime strongly affects the carbon isotope signature  
86 of the plant, with drought increasing  $\delta^{13}\text{C}$  due to low stomatal conductance-driven  $\text{CO}_2$   
87 diffusion (Araus et al., 2003; Condon et al., 2004). However, the effects of other  
88 growing factors such as nitrogen (N) availability on  $\delta^{13}\text{C}$  remain unclear and  
89 contradictory results have been reported. Thus, the  $\delta^{13}\text{C}$  in wheat (*Triticum spp.*) has  
90 been observed either to decrease (Shangguan et al., 2000; Zhao et al., 2007), increase  
91 (Zhao et al., 2007; Serret et al., 2008; Cabrera-Bosquet et al., 2009a) or be unaffected  
92 (Hubick et al., 1990) as N supply increases. Furthermore, the interaction of nitrogen  
93 fertilization and water regime may affect  $\delta^{13}\text{C}$  (Araus et al., 2013).

94

95 During recent years, interest has grown in using oxygen isotope composition ( $\delta^{18}\text{O}$ ) in  
96 plant matter because it integrates evaporative conditions during the crop cycle (Barbour  
97 et al., 2000; Barbour, 2007). It is known that the  $\delta^{18}\text{O}$  of leaf water (and organic matter  
98 that carries leaf water signal) becomes isotopically enriched during transpiration

99 (Barbour and Farquhar, 2000). Indeed, under common environmental conditions (where  
100 the  $\delta^{18}\text{O}$  of ambient vapor, ambient moisture content, and source water do not vary  
101 across different plants), the interest in  $\delta^{18}\text{O}$  is motivated by the concept that  $\delta^{18}\text{O}$  may  
102 be affected by transpiration, which simultaneously depends on stomatal conductance  
103 ( $g_s$ ) (Barbour and Farquhar, 2000; Helliker and Ehleringer, 2002). Similar to  $\delta^{18}\text{O}$ , the  
104 effect of environment on transpiration and evaporation also drives leaf water  
105 evaporative  $^2\text{H}$ -enrichment in the plant (Smith and Freeman, 2006; Feakins and  
106 Sessions, 2010; Kahmen et al., 2013; Cernusak et al., 2016). Therefore, the plant  $\delta^2\text{H}$  in  
107 organic matter is not only influenced by  $g_s$  but also by the effects of climate on  
108 transpiration (Sternberg et al., 1984; Cernusak et al., 2016). Thus, a high correlation  
109 between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in organic matter may indicate source (i.e. water) and  
110 environmental effects (Epstein et al., 1977), whereas a lack of correlation would suggest  
111 either an additional hydrogen (Sternberg et al., 1986) or oxygen (Barbour, 2007) isotope  
112 fractionation effect.

113

114 Theoretically, as driving factors,  $g_s$  and leaf temperature can influence several  
115 parameters of the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  leaf water enrichment model, either directly or  
116 indirectly (Flanagan et al., 1991; Farquhar and Lloyd, 1993). The model relates the  
117 enrichment of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in leaf water above the source of water during evaporation  
118 to (i) the kinetic fractionation during diffusion through the stomata such as  $e_a/e_i$   
119 (through its influence on leaf temperature) (Farquhar et al., 2007), (ii) the Péclet number  
120 (through its influence on transpiration) (Cuntz et al., 2007), (iii) the leaf boundary layer  
121  $\epsilon_k$  (kinetic fractionation that occurs during diffusion and through the pores of the  
122 stomata in the leaf layer), and (iv)  $\epsilon^+$  (the proportional depression of water vapor  
123 pressure by the heavier  $\text{H}_2^{18}\text{O}$  molecule), which is dependent on temperature.

124

125 As indicated above,  $^{18}\text{O}$  is enriched in leaves or other transpiring organs relative to the  
126 source water (Gonfiantini et al., 1965; Farquhar, 1989; Pande et al., 1995). Even so,  
127 diverse factors can affect the use of  $\delta^{18}\text{O}$  to assess plant performance (Barbour and  
128 Farquhar, 2000; Sánchez-Bragado et al., 2016). Thus, the  $\delta^{18}\text{O}$  of photoassimilates may  
129 be affected by the isotopic composition of the water source available to the plant (Yakir  
130 et al., 1990a; Roden et al., 2000; Williams et al., 2005), by the plant height and leaf  
131 length (Helliker and Ehleringer, 2000, 2002) or by fractionation during post-  
132 photosynthetic processes due to biochemical reactions involved in the synthesis of

133 organic matter (Farquhar and Lloyd, 1993) and its subsequent transport within the plant  
134 (Offermann et al., 2011). However, some studies have observed that there is no  
135 fractionation during sucrose transport (Cernusak et al., 2005), although biochemical  
136 fractionation can be impacted by physiological processes such as the carbon turnover  
137 rate, which may affect the  $\delta^{18}\text{O}$  of organic matter (Song et al., 2014). Nonetheless,  $\delta^{18}\text{O}$   
138 has been used to evaluate plant responses to different water regimes in cereals such as  
139 maize (*Zea mays*) and wheat (Barbour et al., 2000; Barbour, 2007; Cabrera-Bosquet et  
140 al., 2009b; Araus et al., 2013). However, studies combining the effects of N supply and  
141 water regime on  $\delta^{18}\text{O}$  are still scarce and the results are contradictory (Cernusak et al.,  
142 2007; Cabrera-Bosquet et al., 2009a; Cabrera-Bosquet et al., 2011; Araus et al., 2013).

143

144 Similar to  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$  in plant organic compounds is affected by the water source  
145 (Epstein et al., 1977; Sternberg et al., 1984; Chikaraishi and Naraoka, 2003; Sachse et  
146 al., 2006; Schwendenmann et al., 2015). However, an important factor that determines  
147 the  $\delta^2\text{H}$  but not the  $\delta^{18}\text{O}$  in plant organic compounds is related to the biochemical  
148 processes between organic compounds and cellular water, which may cause  
149 biosynthetic fractionation of  $^2\text{H}$  (Ziegler et al., 1976; Sternberg et al., 1984; Ziegler,  
150 1989; Yakir and Deniro, 1990; Luo and Sternberg, 1991; Yakir, 1992). Unlike  $\delta^{18}\text{O}$ , the  
151  $\delta^2\text{H}$  of organic matter is also affected by carbon metabolism and it has been proposed,  
152 for example, as a proxy to assess CAM metabolism in plants (Sternberg et al., 1984).  
153 Thus, photosynthesis has a major impact on the  $\delta^2\text{H}$  of plant organic matter (Ziegler et  
154 al., 1976; Luo et al., 1991; Yakir, 1992; Schmidt et al., 2003; Sachse et al., 2012).  
155 Although the mechanisms related to the effects of photosynthetic metabolism on plant  
156  $\delta^2\text{H}$  are insufficiently understood (Sachse et al., 2012), these mechanisms seem clearly  
157 different from those determining  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Thus, the photosynthetic H  
158 fractionation processes that occur during NADPH formation in the photosynthetic light  
159 reactions and triose phosphate primary assimilation may also contribute to determining  
160 the  $\delta^2\text{H}$  in plant organic compounds (Roden et al., 2000). In fact, the NADPH produced  
161 during photosynthesis has been observed as being extremely depleted in  $^2\text{H}$  (Luo et al.,  
162 1991; Schmidt et al., 2003). Moreover, it has been reported that recently produced  
163 autotrophic cellulose in leaves might be depleted in  $^2\text{H}$  compared to available water  
164 (Yakir et al., 1990a; Luo et al., 1991). The reason for such depletion might be related to  
165 reduction reactions, whereby the NADPH-derived hydrogen that is added to carbon  
166 skeletons seems strongly depleted (on average) relative to water (Sachse et al., 2012).

167 Conversely, during heterotrophic metabolism, all other reactions following the primary  
168 assimilation of triose phosphate may enrich the  $^2\text{H}$  of plant organic matter (Roden et al.,  
169 2000) due to the exchange of a large proportion of hydrogen atoms with surrounding  
170 water (Ziegler 1989). In addition, post-photosynthetic  $^2\text{H}$ -fractionation processes may  
171 also occur via the oxidative pentose phosphate pathway during sugar metabolism. Thus,  
172 the NADPH produced may be more enriched (i.e. less depleted) in  $^2\text{H}$  (Yakir and  
173 Deniro, 1990; Schmidt et al., 2003). Hence, photosynthesis depletes the  $^2\text{H}$  of the  
174 carbon-bound hydrogen carbohydrates (fractionation factor around -200‰), whereas  
175 post-photosynthetic metabolism has the opposite effect (+150‰) (Yakir, 1992; Sachse  
176 et al., 2012). Nonetheless, until now there has not been a clear understanding of the  
177 photosynthetic and post-photosynthetic biochemical processes that determine  $\delta^2\text{H}$   
178 fractionation during plant organic biosynthesis. In fact, there have been fewer  
179 applications of hydrogen isotope ratios compared to the other stable light isotopes in  
180 studies of plant organic matter. The underlying reason is related to the presence of  
181 isotopically exchangeable atoms of hydrogen in the organic compounds (oxygen in the  
182 dry matter can also exchange with moisture, although such an effect is predicted to be  
183 much smaller than for  $\delta^2\text{H}$ ) (Yousfi et al., 2013). The percentage of hydrogen atoms of  
184 cellulose that are exchangeable can reach 30% (isotopes of hydrogen bound to oxygen  
185 in hydroxyl groups), whereas the remaining 70% are non-exchangeable hydrogen atoms  
186 bound to carbon (Filot et al., 2006). Therefore, the hydroxyl hydrogen group can easily  
187 exchange with environmental water sources, complicating the interpretation of this  
188 isotope in plant organic matter. Nevertheless, new developments in isotope-ratio mass  
189 spectrometry for compound-specific analyses have promoted the use of H isotopes in  
190 recent years.

191

192 In summary,  $\delta^2\text{H}$  in plants may share some commonalities, in terms of factors affecting  
193 its signature, with  $\delta^{18}\text{O}$  (affected by transpiration and the signature of the source water)  
194 and even with  $\delta^{13}\text{C}$  (through  $g_s$ ), which are both triggered by environmental factors (e.g.  
195 availability of water). However,  $\delta^2\text{H}$  may be further strongly affected by the trophic  
196 (photoautotrophic versus heterotrophic) nature of the plant part considered. In the case  
197 of a leaf (or another photosynthetic organ) the  $\delta^2\text{H}$  in carbohydrates will be a balance  
198 between autotrophic and heterotrophic processes (Yakir et al., 1990b). Therefore,  
199 although there is no evidence that the fractionation effect of  $\delta^2\text{H}$  is associated with

200 environmental stress, the effect of any environmental stress on the photosynthetic  
201 activity might eventually affect the final  $\delta^2\text{H}$  in the carbohydrates of the plant.

202

203 The objective of this study was to evaluate the influence of growing conditions on  
204 transpiration and how these affect the  $\delta^2\text{H}$  of autotrophic (leaves), mixotrophic (ears)  
205 and heterotrophic (roots and mature kernels) organs compared to the  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in  
206 dry matter and the water-soluble fraction in the same organs. For this case study, durum  
207 wheat (*Triticum turgidum* var. *durum*) was chosen due to its frequent exposure to the  
208 vagaries of abiotic stress. Durum wheat is among the main crops cultivated in the  
209 Mediterranean basin (FAOSTAT 2017) where production areas are often  
210 simultaneously exposed to water stress (Lobell et al. 2008) and low nitrogen availability  
211 (Oweis et al., 1998; Sadras, 2004). Moreover, there is increasing evidence that ongoing  
212 climate change is already stagnating productivity (Moore and Lobell, 2015; Ceglar et  
213 al., 2016) by decreasing precipitation while increasing evapotranspiration. Thus, a panel  
214 of modern cultivars and landraces of durum wheat were grown in the field during two  
215 consecutive years under different combinations of water and nitrogen fertilization. To  
216 the best of our knowledge there have been no field studies in crop species reporting on  
217 the variation in  $\delta^2\text{H}$  within different organs and among genotypes under a combination  
218 of different water and nitrogen conditions and therefore comparisons among these three  
219 stable light isotopes ( $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{18}\text{O}$ ) as ecophysiological indicators of plant performance  
220 are absent.

221

222

## 223 **RESULTS**

224 Average grain yield (*GY*) including all growing conditions was higher in 2011 (3.1  
225  $\text{Mg}\cdot\text{ha}^{-1}$ ) compared to that in 2010 (1.7  $\text{Mg}\cdot\text{ha}^{-1}$ ) (data not shown). Similarly, cultivars  
226 showed higher *GY* (1.9  $\text{Mg}\cdot\text{ha}^{-1}$  and 3.1  $\text{Mg}\cdot\text{ha}^{-1}$  for 2010 and 2011, respectively)  
227 compared to that in landraces (1.5  $\text{Mg}\cdot\text{ha}^{-1}$  and 1.6  $\text{Mg}\cdot\text{ha}^{-1}$  for 2010 and 2011,  
228 respectively) during both growing seasons. Moreover, the *GY* of both landraces and  
229 cultivars was higher under support irrigation (SI) than rainfed (RF) conditions (Tables 1  
230 and Table 2). Furthermore, whereas  $g_s$  was much higher under SI compared to that  
231 under RF conditions in 2010 and 2011 (Table 1 and Table 2), no significant differences  
232 were observed between landraces and cultivars in 2010 (Table 1). In contrast, in 2010,  
233  $g_s$  decreased in response to nitrogen fertilization (Table 1).

234

235 *Hydrogen, oxygen and carbon isotope composition across tissues*

236 Mean values averaged across genotypes of stable hydrogen ( $\delta^2\text{H}$ ), oxygen ( $\delta^{18}\text{O}$ ) and  
237 carbon ( $\delta^{13}\text{C}$ ) isotope composition within different tissues are shown in Figure 1.  
238 Hydrogen isotopic composition in mature grains showed the most enriched (less  
239 negative) values ( $\delta^2\text{H}_{\text{grain}}=-32.4\text{‰}$ ) compared to that in the ears ( $\delta^2\text{H}_{\text{earDM}}=-92.4\text{‰}$ ),  
240 flag leaves ( $\delta^2\text{H}_{\text{flagDM}}=-115.2\text{‰}$ ) and roots ( $\delta^2\text{H}_{\text{rootsDM}}=-67.0\text{‰}$ ). For the  $\delta^{13}\text{C}$ , the  
241 ears ( $\delta^{13}\text{C}_{\text{ear DM}}=-24.7\text{‰}$ ) and mature grains ( $\delta^{13}\text{C}_{\text{grain}}=-24.3\text{‰}$ ) showed the most  
242 enriched values compared to that in the flag leaves ( $\delta^{13}\text{C}_{\text{flagDM}}=-25.7\text{‰}$ ) (Fig. 1). In the  
243 case of  $\delta^{18}\text{O}$ , the most enriched tissue was the flag leaf ( $\delta^{18}\text{O}_{\text{flagDM}}=30.6\text{‰}$ ).

244

245 Hydrogen isotope composition of stem water ( $\delta^2\text{H}_{\text{stemW}}=-45.9\text{‰}$ ) was depleted  
246 compared to that of the grain DM, but enriched compared to that of the flag leaves  
247 (WSF), the ears (WSF) and the roots (DM) (Fig. 2). In contrast, the  $\delta^{18}\text{O}$  of stem water  
248 ( $\delta^{18}\text{O}_{\text{stemW}}=-5.6\text{‰}$ ) displayed the most depleted value regardless of the tissues and  
249 fractions (DM, WSF) analyzed (Fig. 2). Moreover, the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of stem water were  
250 more depleted compared to that of grain water ( $\delta^2\text{H}_{\text{grainW}}=-15.3\text{‰}$  and  $\delta^{18}\text{O}_{\text{grainW}}=7.0\text{‰}$ )  
251 and flag leaf water ( $\delta^2\text{H}_{\text{flagW}}=9.2\text{‰}$  and  $\delta^{18}\text{O}_{\text{flagW}}=11.3\text{‰}$ ) (Fig. 2).

252

253 *Fractionation of hydrogen, oxygen and carbon isotope composition across plant tissues*

254 In order to further assess whether similar fractionation processes affected isotopic  
255 composition of hydrogen, oxygen and carbon within the plant, correlation analysis was  
256 performed between different isotope compositions ( $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$ ) in the WSF of  
257 the same plant tissue (mature kernels, ears and flag leaves) (Fig. 3). The strongest  
258 relationship in the flag leaf WSF (left columns, Fig. 3) was observed between  $\delta^{18}\text{O}$  and  
259  $\delta^{13}\text{C}$  ( $r = 0.87$ ,  $P < 0.001$ ), whereas in the WSF of the ears the strongest correlation was  
260 found between  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  ( $r = 0.74$ ,  $P < 0.001$ ) followed by  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  ( $r = 0.65$ ,  
261  $P < 0.001$ ). In mature kernels,  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  were highly correlated ( $r = 0.70$ ,  $P < 0.001$ ),  
262 whereas  $\delta^{18}\text{O}$  did not correlate with either  $\delta^2\text{H}$  or  $\delta^{13}\text{C}$ .

263

264 In order to estimate whether the fractionation processes affecting  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  were  
265 similar in the water transported by different plant tissues, correlation analysis was  
266 performed between the oxygen and hydrogen isotope compositions of the water  
267 extracted from different tissues (Table 3).  $\delta^2\text{H}_{\text{flagW}}$  was positively correlated with



268  $\delta^2\text{H}_{\text{grainW}}$  ( $r=0.66$ ,  $P<0.001$ ), whereas no correlation was observed with  $\delta^2\text{H}_{\text{stemW}}$ .  
269 Similarly,  $\delta^{18}\text{O}_{\text{flagW}}$  was positively correlated with  $\delta^{18}\text{O}_{\text{grainW}}$  ( $r=0.67$ ,  $P<0.001$ ) but not  
270 with  $\delta^{18}\text{O}_{\text{stemW}}$ . In addition, in order to estimate whether the same fractionation  
271 processes affected  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in the water of tissues, correlation analyses between the  
272  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of the water in the same tissues were performed (Table 3). It was observed  
273 that the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in flag leaf water were strongly correlated ( $r=0.99$ ,  $P<0.001$ ).  
274 Similarly,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  were strongly correlated in the grain water ( $r=0.99$ ,  $P<0.001$ )  
275 and stem water ( $r=0.81$ ,  $P<0.001$ ).

276

#### 277 *Water and nitrogen effects on carbon, oxygen and hydrogen isotope composition*

278 Significant differences within SI and RF conditions (Table 1) were mainly observed in  
279  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  in 2010. Concerning  $\delta^{18}\text{O}$ , only the flag leaf DM or the flag leaf WSF  
280 showed significant differences between the two water regimes in 2010. Overall, water  
281 stress tended to increase  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  irrespective of the tissue or fraction  
282 analyzed, with the exception of  $\delta^2\text{H}_{\text{rootsDM}}$  (Table 1). Furthermore, HN plants showed  
283 higher  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  compared that in to LN plants, although no significant differences  
284 were observed in  $\delta^2\text{H}_{\text{rootsDM}}$ ,  $\delta^2\text{H}_{\text{stemW}}$  and  $\delta^{13}\text{C}_{\text{flagWSF}}$ . By contrast,  $\delta^{18}\text{O}$  did not  
285 exhibit significant differences among fertilization conditions, with the exception of in  
286 the roots ( $\delta^2\text{H}_{\text{rootsDM}}$ ).

287

#### 288 *Carbon, oxygen and hydrogen isotope composition in cultivars and landraces*

289 Overall, the  $\delta^2\text{H}$  in 2010 was lower in landraces compared to cultivars (Table 1),  
290 although significant differences were only observed in the  $\delta^2\text{H}$  of the WSF of the flag  
291 leaf and ear. A similar trend was exhibited by  $\delta^{13}\text{C}$ , with landraces having lower  $\delta^{13}\text{C}$   
292 compared to cultivars, with the exception of  $\delta^{13}\text{C}_{\text{flagDM}}$  (Table 1). Conversely,  $\delta^{13}\text{C}_{\text{grain}}$   
293 and  $\delta^2\text{H}_{\text{grain}}$  were less enriched in landraces compared to cultivars. There were no  
294 significant differences in  $\delta^{18}\text{O}$ , regardless of the organs and fractions considered, with  
295 the exception of in the grains (Table 1).

296

#### 297 *Correlations of $\delta^2\text{H}$ , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ with GY, $g_s$ and N content*

298 The  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  in DM and WSF from different tissues plus mature kernels were  
299 correlated with GY,  $g_s$  and N content in the flag leaves (N-Flag) and the ears (N-Ear).  
300 Correlations were calculated including all genotypes and either the whole set of growing  
301 conditions in 2010 (Fig. 4), or across different water regimes for a given nitrogen

302 fertilization level (HN and LN) or across nitrogen regimes within each water condition  
303 (SI and RF) (Table 4). In general,  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  in the different tissues and water were  
304 negatively correlated with  $GY$  ( $P<0.05$ ) and  $g_s$  ( $P<0.01$ ) when all genotypes and  
305 growing conditions were combined (Fig. 4) and across the four different combinations  
306 of water and nitrogen regimes (SI, RF, HN, LN) (Table 4), with  $\delta^{13}\text{C}$  in mature grains  
307 showing the highest correlation against  $GY$  when all growing conditions were included  
308 (Fig. 4). Conversely,  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  were positively correlated with N-Flag ( $P<0.01$ ) and  
309 N-Ear ( $P<0.01$ ) under SI and RF conditions (Table. 4), whereas under HN conditions,  
310 this correlation was negative (Table 4). With regard to  $\delta^{18}\text{O}$ , it was marginally  
311 correlated with  $GY$ ,  $g_s$ , N-Flag and N-Ear in 2010 (Fig. 4 and Table 4). However, when  
312 all growing conditions were combined in 2011 (Table 3),  $GY$  was negatively and  
313 strongly correlated with  $\delta^{18}\text{O}_{\text{flagDM}}$  ( $P<0.001$ ) as well as with  $\delta^{18}\text{O}_{\text{flagW}}$  ( $P<0.001$ ).  
314 Additionally, correlations of  $\delta^{18}\text{O}_{\text{grainW}}$  with  $GY$  were also observed ( $P<0.001$ ) (Table  
315 3).

316

317 Furthermore, in order to test which isotope, tissue and fraction better explained yield, a  
318 stepwise regression analysis was performed in the 2010 trials between the analyzed  
319 signatures of the different isotopes, tissues and fractions (either DM and WSF) as  
320 independent variables and  $GY$  as the dependent variable (Table 5). The stepwise  
321 analysis was performed combining all treatments together (global) for each of the water  
322 regimes and both fertilization levels together (SI, RF) and for each nitrogen fertilization  
323 level and both water regimes combined (HN, LN). In the global and HN analyses, the  
324 first independent variable chosen by the model was  $\delta^{13}\text{C}_{\text{grain}}$ , whereas in the LN  
325 analysis, it was the  $\delta^{18}\text{O}_{\text{flagWSF}}$ . Conversely, in the SI and RF analyses,  $\delta^2\text{H}_{\text{earWSF}}$  and  
326  $\delta^2\text{H}_{\text{grain}}$  were the first variables chosen by the model, respectively.

327

### 328 *Experimental estimation of the ETR's association with $\delta^2\text{H}$ depletion*

329 The  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$ , together with stomatal conductance ( $g_s$ ) and electron transport rate  
330 (ETR), were assessed in the flag leaves of the same durum wheat variety growing under  
331 controlled conditions under two different relative humidity (RH) conditions (40% and  
332 80% RH). Plants growing under 80% RH showed depleted  $\delta^{13}\text{C}$  values and higher  
333 stomatal conductance in the flag leaves compared to that in plants grown under 40%

334 RH. Accordingly, flag leaves exhibited more depleted  $\delta^2\text{H}$  values and higher ETR under  
335 80% RH compared to that under 40% RH (Table S1).

336

## 337 **DISCUSSION**

338

### 339 *Photosynthetic fractionation and autotrophic effects*

340 Similar to the effect on  $\delta^{13}\text{C}$ , it has been reported that photosynthesis could have a  
341 major impact on  $\delta^2\text{H}$  (Sternberg et al., 1984). In our study, the  $\delta^2\text{H}$  from the flag leaf  
342 water showed enriched values compared to that of  $\delta^2\text{H}_{\text{flag}}$  (either DM or WSF),  
343 indicating that depleted values of the  $\delta^2\text{H}_{\text{flag}}$  in plant matter or the water-soluble fraction  
344 may not originate from evaporative processes, but are mainly due to photosynthetic  
345 reactions. In fact,  $\delta^2\text{H}_{\text{flag}}\text{WSF}$  vs  $\delta^{13}\text{C}_{\text{flag}}\text{WSF}$  were better correlated than  $\delta^2\text{H}_{\text{flag}}\text{WSF}$  vs  
346  $\delta^{18}\text{O}_{\text{flag}}\text{WSF}$  (Fig. 3), suggesting that leaf  $\delta^2\text{H}$  is not only affected by changes in  
347 transpiration and stomatal conductance, alongside the evaporative conditions (Cernusak  
348 et al., 2016), but also by photosynthetic reactions (Yakir et al., 1990b) and carbon  
349 metabolism in plants (Cormier et al., 2018). Recent autotrophically-produced cellulose,  
350 lipids (Sternberg, 1988) or starch (Hayes, 2001) in leaves might be depleted in  $^2\text{H}$   
351 compared to the available water (Yakir et al., 1990a) (Fig. 2). Although the hydrogen  
352 isotope composition in the leaf plant water may be imprinted in sugars and metabolites  
353 and thus also retained in organic compounds (Cernusak et al., 2016), the isotopic  
354 composition of the H transferred from NADPH to biosynthetic substrates might be one  
355 of the most important factors controlling the hydrogen-isotopic composition of organic  
356 matter in photosynthetic organs (Hayes, 2001). This evidence was supported and  
357 quantified by a study performed by Yakir and Deniro (1990) in *Lemma gibba* L. grown  
358 under autotrophic conditions. In this study, the negative fractionation factor between the  
359 water and photosynthates caused a strong depletion (-171‰). Such a low delta value  
360 was postulated to be the consequence of the extremely deuterium-depleted protons (Luo  
361 et al., 1991; Hoganson and Babcock, 1997) used (from a water molecule within the cell)  
362 for the reduction of  $\text{NADP}^+$  to NADPH. However, in a study performed by Cormier et  
363 al. (2018) in different vascular plant species, the increase in light intensity (above 115  
364  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and consequently the photosynthetic rate, did not clearly deplete the  $\delta^2\text{H}$   
365 in the studied organic compounds. In spite of that, H pools that are strongly depleted  
366 relative to leaf water have been observed to result from photosynthetic  $^2\text{H}$  fractionation

367 in the chloroplast during light reactions where ferredoxin-NADP<sup>+</sup> reductase produces  
368 NADPH with reduced H (Luo et al., 1991). The results observed in the growth chamber  
369 experiment, with plants grown under two different relative humidity (RH) conditions  
370 (40% and 80% RH), agree with the findings of Luo et al. (1991) (Table S1). Plants  
371 growing under 80% RH showed depleted  $\delta^{13}\text{C}$  values and higher stomatal conductance  
372 in the flag leaf, suggesting that these plants were exposed to less water-limiting  
373 conditions compared to that for plants grown under 40% RH. Accordingly, flag leaves  
374 exhibited more depleted  $\delta^2\text{H}$  values and higher electron transport rates (ETR) under  
375 80% RH compared to that under 40% RH (Table S1). Because the ETR is associated  
376 with a reduction of NADP<sup>+</sup> to NADPH during the light reaction of photosynthesis  
377 (Foyer et al., 2012) under less water-limiting conditions (80% RH), it is worth  
378 considering that there is a causal association of the ETR with the contribution of  $\delta^2\text{H}$ -  
379 depleted NADPH to organic  $\delta^2\text{H}$ . In contrast, under more water-limiting conditions  
380 (40% RH), the ETR was lower, causing a reduction in NADPH levels and consequently  
381 a decrease in the contribution of  $\delta^2\text{H}$ -depleted NADPH to organic  $\delta^2\text{H}$ , resulting in  
382 enriched plant organic  $\delta^2\text{H}$  compared to 80% RH. Indeed, the depleted values of  $\delta^2\text{H}$   
383 observed in our experiment in the flag leaves and ears compared to that in the grains  
384 would agree with the autotrophic activity of the former organs (Yakir and Deniro,  
385 1990).

386

#### 387 *Evaporative fractionation: transpirative effects*

388 The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of water from the flag leaves, ears and grains were enriched  
389 compared to the source of water (water collected from the base of the stem). Indeed, the  
390 isotope signature of the water from different plant tissues might influence the isotope  
391 signature in the DM and WSF. In our study the increase in the  $\delta^2\text{H}$  and the  $\delta^{18}\text{O}$  of the  
392 DM and WSF from the flag leaves to the apical part of the plant (flag leaves compared  
393 to the ears and grains) may be due in part to the effect of a progressive enrichment in  
394  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of the plant water associated with evaporative demand (Helliker and  
395 Ehleringer, 2002). Likewise,  $\delta^{18}\text{O}_{\text{flag}}\text{WSF}$  and  $\delta^{13}\text{C}_{\text{flag}}\text{WSF}$  (and  $\delta^{18}\text{O}_{\text{ear}}\text{WSF}$  and  
396  $\delta^{13}\text{C}_{\text{ear}}\text{WSF}$ ) were strongly correlated, suggesting that in autotrophic organs (leaves and  
397 to some extent ears) both isotopes are probably governed by changes in transpiration  
398 and stomatal conductance, as previously reported in durum wheat (Cabrera-Bosquet et  
399 al., 2009a).

400

401 However, in spite of the previously discussed evaporation-driven effect from the bottom  
402 to the top of the aerial parts of the plant, the grain water was less enriched in  $\delta^2\text{H}$  and  
403  $\delta^{18}\text{O}$  compared to that in leaf water, although the grain water was enriched compared to  
404 that in the source water. In fact, leaf water (and organic matter that carries the leaf water  
405 signal) becomes more  $^2\text{H}$  enriched than the grains as a result of the evaporative process  
406 during transpiration (Gonfiantini et al. 1965; Craig and Gordon 1965). In the case of  
407 water in the grains, different mechanisms may apply. Even if the grains have the  
408 photosynthetic “green layer” of aleurone (Caley et al., 1990), few stomata are present on  
409 the pericarp (Barlow et al., 1980). In addition, grains are surrounded by the ear bracts,  
410 which may therefore minimize transpirative losses (Bort et al., 1996). Moreover,  
411 transpiration of the culm, including leaf sheaths is smaller than in the leaf blades, which  
412 may also cause less isotopic enrichment of the water if it goes straight from the base of  
413 the stem to the growing grains than for example from the base of the stem to the leaf  
414 blades (Araus and Tapia, 1987). In addition, there is a xylem discontinuity in the floral  
415 axis (Cochrane and Duffus, 1979) and therefore in the longer-term water transport from  
416 the stem base to the growing grains. Taking into consideration that transpirative water  
417 losses in the grain are low, the acropetal transport of leaf water in the phloem  
418 to the developing grains may contribute to enrichment of the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in the grains  
419 relative to the base of the stem as a result of mixing of the phloem water from the leaves  
420 with the source water from the base of the stem (Cernusak et al., 2016). In addition, the  
421 biphasic enrichment in the grains linked to developmental metabolism of the grain and  
422 rapid loss of water during the last part of the grain filling might have enriched the  $^2\text{H}$  in  
423 the grain water compared to that in the source of water (Pande et al., 1994). Indeed,  
424 invoking variation in the  $\delta^2\text{H}$  of water among tissues as an explanation for among-tissue  
425 (leaf versus grain) variation in dry matter and the WSF does not seem a convincing  
426 conclusion. Further, the “progressive enrichment” concept (Helliker and Ehleringer,  
427 2002) does not appear to hold at the whole-plant level (or at least when comparing  
428 leaves with grains).

429 Moreover, evaporative  $^2\text{H}$  enrichment of leaf water was markedly higher compared to  
430  $^{18}\text{O}$  enrichment. The contrast between the  $^2\text{H}$  and  $^{18}\text{O}$  may be related to the relative  
431 magnitude of the equilibrium and kinetic fractionation in the Craig and Gordon model  
432 of evaporative site enrichment (Cernusak et al., 2016). The  $^{18}\text{O}$  in the leaf water is  
433 mainly triggered by kinetic fractionation, whereas equilibrium fractionation mainly

434 dominates  $^2\text{H}$  (Cernusak et al., 2016). Kinetic effects are closely dependent on stomata  
435 and boundary layer resistance (Farquhar et al., 1989), whereas the equilibrium effect  
436 varies as a function of temperature (Bottinga and Craig, 1968). In a study performed in  
437 Australia by Kahmen et al. (2013) over a large-scale environmental gradient, the effect  
438 of disequilibrium between the source water and atmospheric vapor was stronger for  $^2\text{H}$   
439 than that for  $^{18}\text{O}$  (correlations between the leaf water isotope signature and air relative  
440 humidity were stronger for  $\delta^{18}\text{O}$  than that for  $\delta^2\text{H}$ ). Moreover, under non-equilibrium  
441 conditions, evaporative processes tend to cause greater (relative) enrichment of  $^{18}\text{O}$  than  
442  $^2\text{H}$ , and as a consequence the slope between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of water gets flatter  
443 (Dansgaard, 1964). However, plants were exposed under steady-state conditions  
444 because leaves exhibit relatively open stomata during the day (considering that leaves  
445 were not succulent) (Cernusak et al., 2008) and therefore these could be assumed as  
446 equilibrium conditions. Under such conditions, isotopic enrichment is likely to be  
447 stronger for  $^2\text{H}$  in the leaf water compared to  $^{18}\text{O}$ , causing proportionally higher values  
448 in  $^2\text{H}$  than  $^{18}\text{O}$  in the leaf water.

449

#### 450 *Post-photosynthetic fractionation and heterotrophic effects*

451 During heterotrophic metabolism a substantial fractionation of hydrogen isotopes from  
452 leaf water to organic matter has been described (Sternberg et al., 1986), which may lead  
453 to  $^2\text{H}$  enrichment in organic matter (Ziegler, 1989). Post-photosynthetic  $^2\text{H}$  enrichment  
454 starts in the rapid reciprocal exchange between the triose phosphate pool and the  
455 hexosephosphate pool during carbohydrates synthesis (Buchanan et al., 2015; Cormier  
456 et al., 2018). Subsequently, there are a number of processes that lead to  $^2\text{H}$ -enrichment  
457 of carbohydrates: (i) the synthesis of glyceraldehyde-3-phosphate in the Calvin cycle,  
458 which enables exchange with cellular ( $^2\text{H}$ -enriched) water (Rieder and Rose, 1959); (ii)  
459 the C-bound H in newly photosynthesized glyceraldehyde-3-phosphate derives from an  
460  $^2\text{H}$ -enriched precursor molecule, 3-phosphoglyceraldehyde (due to previous exchange  
461 with cellular water); (iii) the formation of fructose 1,6-biphosphate from two triose  
462 phosphates during hexosephosphate production leads to loss of one of the four C-bound  
463 H atoms to the nearby water (Rieder and Rose, 1959), and results in an  $^2\text{H}$ -enrichment  
464 in the glyceraldehyde-3-phosphate pool due to the more rapid reaction of the lighter  
465 isotopologues during this process (Schmidt et al., 2015); and (iv) the interconversion of  
466 glucose 6-phosphate and fructose 6-phosphate, which is performed by the enzyme

467 phosphoglucose isomerase, may also  $^2\text{H}$ -enrich glyceraldehyde-3-phosphate pools  
468 (Schleucher et al., 1999).

469

470 According to our results, the  $\delta^2\text{H}$  of mature grains was enriched compared to that in  
471 other analyzed plant parts (including the ear) (Fig. 2), and it was also enriched in roots  
472 compared to that in flag leaves (Table 1). Only 15% of C-bound H atoms present in  
473 carbohydrates in heterotrophic tissues originate from the  $^2\text{H}$ -depleted NADPH that is  
474 produced during the light reactions of photosynthesis in the chloroplast (Cormier et al.,  
475 2018), and this leads to  $^2\text{H}$ -enriched values in heterotrophic organs such as grains and  
476 specific compounds such as starch or cellulose (Epstein et al., 1976; Sternberg et al.,  
477 1984). Such findings suggest that  $\delta^2\text{H}$  was exposed to post-photosynthetic enrichment  
478 (as explained above) in most heterotrophic organs (such as the grains and roots) in  
479 comparison to the more depleted  $\delta^2\text{H}$  in autotrophic organs such as the leaves (Yakir et  
480 al., 1990b).

481

482 If we follow the same reasoning used for the leaves but with respect to the ear, the  $\delta^2\text{H}$   
483 in the WSF of the ears was depleted compared to mature grains, but enriched compared  
484 to the flag leaves (Fig. 2). On one hand, such increases from the flag leaves to the ears  
485 could be a consequence of lower  $g_s$  in the latter organ (Araus et al., 1993; Tambussi et  
486 al., 2005) as well as the result of the mixotrophic nature of the ear bracts (combining  
487 large portions of heterotrophic areas with photosynthetic tissues) compared to the leaves  
488 (Blum, 1985; Knoppik et al., 1986; Araus et al., 1993; Bort et al., 1994; Li et al., 2006).  
489 Furthermore, enriched  $\delta^2\text{H}$  values of the ears in the WSF compared to that in the flag  
490 leaves could be a consequence of a degree of CAM metabolism in the ears (Sternberg  
491 and Deniro, 1983; Sternberg et al., 1984; Winter et al., 2008; Feakins and Sessions,  
492 2010; Sachse et al., 2012; Winter and Holtum, 2014). Recent studies in wheat glumes  
493 and lemmas have shown that the activity of the RuBP carboxylase enzyme decreases  
494 significantly in response to water stress, whereas the activity of phosphoenolpyruvate  
495 carboxylase increases along with that of NADP-malate dehydrogenase (Jia et al., 2015).

496

497 On the other hand, such a decrease in the  $\delta^2\text{H}$  in the DM of the ears with respect to the  
498 grains (besides the fact that the grains are subjected to heterotrophic metabolism) could  
499 be due to the presence of epicuticular waxes in the ears alongside the support tissues  
500 (Araus et al., 1993). In fact, it has been reported in a deciduous conifer that lipids

501 derived from epicuticular waxes and support tissues (e.g. collenchyma and  
502 sclerenchyma) are highly depleted in deuterium (Sessions et al., 1999; Chikaraishi et al.,  
503 2004; Hou et al., 2007; Yang and Leng, 2009; Zhou et al., 2011) because  $^2\text{H}$ -depleted  
504 NADPH is a critical source of H in lipid biosynthesis (Cormier et al., 2018).  
505 Consequently, the presence of lipids derived from cuticular waxes in the DM of the ears  
506 might have depleted the  $\delta^2\text{H}$  compared to that in the grains.

507

#### 508 *Water and nitrogen effects on $\delta^{13}\text{C}$ , $\delta^{18}\text{O}$ and $\delta^2\text{H}$*

509 Grain yield (GY) recorded during 2010 and 2011 was in the range reported in earlier  
510 work in the Mediterranean basin under dry rainfed and low supplementary irrigation  
511 conditions (Araus et al., 1998, 2003, 2013). Support irrigation significantly increased  
512 yield, whereas no effect was observed on yield under nitrogen fertilization conditions  
513 (Table 1).

514

515 In our study, N fertilization increased  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  in the leaf DM, whereas  $\delta^{18}\text{O}$   
516 showed a similar trend, although no significant differences were observed. Likewise, an  
517 increase in  $\delta^{13}\text{C}$  due to N fertilization has been reported (Farquhar, 1989) as a  
518 consequence of a reduction in the  $C_i/C_a$  ratio, due to either an increase in photosynthetic  
519 capacity or a decrease in  $g_s$  (Farquhar and Richards, 1984; Condon et al., 2004). Such  
520 findings agree with our results, where fertilized plots showed lower  $g_s$  compared to that  
521 of non-fertilized plots (Table 1). Moreover, the higher  $\delta^2\text{H}$  values in the leaf dry matter  
522 under N fertilization can be explained by the evaporative  $^2\text{H}$  enrichment of leaf water.  
523 In the modified Péclet effect model (Farquhar and Lloyd, 1993), transpiration has been  
524 observed to reduce  $^2\text{H}$ -enrichment of leaf water due to a mixture of leaf water and  
525 source of water (Cernusak et al., 2016). Therefore, the increase in  $\delta^2\text{H}$  in the leaf DM  
526 under N fertilization observed in our experiments can be explained by reduced  $g_s$  and a  
527 subsequent decrease in transpiration resulting in a decreased Péclet effect (Cernusak et  
528 al., 2016). In addition,  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  were positively correlated with N-Flag (nitrogen  
529 content of the flag leaves DM) and negatively correlated with  $g_s$  under RF and SI  
530 conditions (both including HN and LN treatments) (Table 4). Such results indicate that  
531 biomass is increased with increases in nitrogen supply, forcing the plants to compete for  
532 water resources and exacerbating water stress (therefore resulting in lower  $g_s$  and higher  
533  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$ ). However, under SI conditions, N fertilization (which may have increased  
534 growth and consequently biomass and yield) did not increase N-Flag as a consequence



535 of a growth dilution effect in the leaf due to leaf expansion (Salazar-Tortosa et al.,  
536 2018). This is supported by the negative correlation between *GY* and N-Flag ( $r=-0.426$ ,  
537  $P<0.01$ , data not shown) under SI conditions, whereas under RF conditions the  
538 correlation was positive ( $r=0.285$ ,  $P<0.05$ , data not shown). Thus, N fertilization under  
539 SI conditions showed positive correlation between  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  and N-Flag, supporting  
540 the idea that plants with lower N-Flag showed higher *GY* (i.e biomass), which as  
541 mentioned before may have caused a temporary mild water stress during plant growth.

542 However, under HN conditions (but including the two water regimes) correlations of  
543  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  with N-Flag were in some cases negative (Table 4). These results suggest  
544 that under HN conditions (including SI and RF regimes), N fertilization does not  
545 necessarily have a negative effect as water stress increases, but rather, the opposite is  
546 observed. It has been reported that providing N when there is water available in the soil  
547 (i.e. under irrigation conditions) may improve not only growth but also the water status  
548 of the crop by contributing to better root growth (Jensen et al., 1990). Overall, these  
549 findings suggest that  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  are subject, at least in part, to a similar source of  
550 variation; meaning that both isotopes responded with an increase in isotope signature as  
551 a result of nitrogen fertilization for a given level of water regime (SI, RF) or with a  
552 decrease in isotope signature in response to water supply under nitrogen fertilization  
553 conditions (HN).

554

#### 555 *Differences in $\delta^{13}\text{C}$ , $\delta^{18}\text{O}$ and $\delta^2\text{H}$ between cultivars and landraces*

556 *GY* was higher in cultivars compared to that in landraces for both growing years of the  
557 study. Although landraces in this study were chosen on the basis of their close  
558 phenology to modern cultivars, the latter on average still reached heading 5 days earlier  
559 (data not shown). Cultivars have been observed as having a shorter duration to heading  
560 compared to landraces (Araus et al., 2002, 2013), escaping from water stress produced  
561 during the reproductive stage (Araus et al., 2007). In fact, more enriched values of  $\delta^{13}\text{C}$ ,  
562  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  in grains were observed in landraces compared to that in cultivars (Table  
563 1), evidencing that landraces were exposed to an extended stress episode contributing to  
564 a lower *GY* compared to cultivars (Araus et al., 2007, 2013).

565

#### 566 *Applicability of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for assessing plant performance*

567 In agreement with previous studies (Condon et al., 1987; Araus et al., 1998; Fischer et  
568 al., 1998; Araus et al., 2003; Monneveux et al., 2005; Lopes and Reynolds, 2010),  $\delta^{13}\text{C}$   
569 was negatively correlated with *GY* when all growing conditions were combined (Fig. 4).  
570 Conversely, some correlations of  $\delta^{13}\text{C}$  with *GY* under SI conditions (including HN and  
571 LN conditions) were positive for the flag leaves and the ears and negative for the grains  
572 (Table 4). The positive slope between  $\delta^{13}\text{C}$  (in the flag leaves and ear) and *GY* under SI  
573 may be the consequence of N fertilization causing water stress as discussed above.  
574 Moreover, negative genotypic correlations with *GY* were only observed with  $\delta^{13}\text{C}_{\text{grain}}$   
575 (Table S2) and were weaker in 2010 than in 2011. Indeed, correlations between *GY* and  
576  $\delta^{13}\text{C}_{\text{grain}}$  decreased under poor growing conditions (Fig. S1), whereas the  $\delta^{18}\text{O}_{\text{flag}}$  WSF  
577 showed the opposite trend (Fig. S1). According to our results, in trials under drought  
578 conditions with mean yields below  $2 \text{ Mg}\cdot\text{ha}^{-1}$  (as was the case for 2010), non-significant  
579 (Araus et al., 2003) or even positive relationships between *GY* and  $\delta^{13}\text{C}_{\text{grain}}$  (Voltas et  
580 al., 1999) have been reported, suggesting that higher plant WUE (and thus higher  $\delta^{13}\text{C}$ )  
581 increases yield under stress (Farquhar and Richards, 1984; Araus et al., 2003, 2013;  
582 Condon et al., 2004). Thus,  $\delta^{13}\text{C}_{\text{grain}}$  could be a good indicator of the water strategy that  
583 plants are following.

584

585 Concerning  $\delta^{18}\text{O}$  in 2011, the isotope composition in the grain ( $\delta^{18}\text{O}_{\text{grain}}$ ) was strongly  
586 associated with *GY* ( $r=-0.83$ ,  $P<0.000$ ) under all water regimes and nitrogen levels  
587 combined (Table 3). However, the correlation was weaker in 2010 ( $r=-0.21$ ,  $P=0.027$ ,  
588 Fig.4). The lack of consistency between the 2010 and 2011 crop seasons may be related  
589 to differences in environmental conditions. *GY* was much lower in 2010 ( $1.7 \text{ Mg}\cdot\text{ha}^{-1}$  on  
590 average) compared to that in 2011 ( $3.1 \text{ Mg}\cdot\text{ha}^{-1}$  on average). In fact, in a study in wheat  
591 by Barbour et al., (2000), correlations of  $\delta^{18}\text{O}_{\text{grain}}$  with *GY* and  $g_s$  were also not constant  
592 among the three seasons they analyzed. In the same study,  $\delta^{18}\text{O}_{\text{grain}}$  was only correlated  
593 with *GY* during one season, and it was the season with the highest precipitation and  
594 lowest solar radiation. Such results indicate that  $\delta^{18}\text{O}_{\text{grain}}$  might not reflect evaporative  
595 conditions under narrow environmental ranges and with moderate to severe drought.  
596 Therefore, as a consequence of high levels of remobilization under more severe water  
597 conditions, preservation of evaporative conditions imprinted in the  $\delta^{18}\text{O}$  of grains might  
598 be low or even non-existent (Barbour et al., 2000; Ferrio et al., 2007). Likewise, the  
599 disparity observed in our study between the two growing seasons regarding the  
600 relationship between  $\delta^{18}\text{O}_{\text{grain}}$  and *GY* could be due to the relative proportions of

601 remobilized photo-assimilates (Barbour et al., 2000). Triose phosphates formed from  
602 photosynthesis during the day are converted to sucrose for transport (Barbour and  
603 Farquhar, 2000). Thus, the main exchange of water with carbonyl oxygen occurs in the  
604 leaves during the formation of triose phosphate molecules because two of the three  
605 oxygen atoms present in the molecule belong to carbonyl groups (Sternberg et al., 1986;  
606 Barbour et al., 2000). Indeed, correlations of *GY* with  $\delta^{18}\text{O}_{\text{flag}}$  in the WSF and DM were  
607 higher than with  $\delta^{18}\text{O}_{\text{grain}}$  (Fig.4 and Fig.S2), indicating that signals of the evaporative  
608 conditions are still preserved in leaf assimilates but not in other organs because no  
609 correlations of *GY* with the  $\delta^{18}\text{O}_{\text{ear}}$  (either DM or WSF),  $\delta^{18}\text{O}_{\text{grain}}$  or  $\delta^{18}\text{O}_{\text{roots}}$  were  
610 observed. On one hand, as mentioned above, such a lack of correlation may be related to  
611 the  $\delta^{18}\text{O}$  fractionation associated with biochemical reactions during the synthesis of  
612 organic matter (Farquhar and Lloyd, 1993) and its subsequent transport (Offermann et  
613 al., 2011). On the other hand, the  $\delta^{18}\text{O}$  of organic matter may also be influenced by the  
614 source of water (Fig. 1 and Fig. 2) (Epstein et al., 1977; Yakir et al., 1990b; Roden et  
615 al., 2000; Williams et al., 2005; Barbour, 2007). This is not straightforward because the  
616  $\delta^{18}\text{O}$  of source water (water from the base of the stem) is also subjected to evaporative  
617 enrichment in the leaf during transpiration (Farquhar et al., 1993) and during grain  
618 formation (Pande et al., 1994) (Fig. 2). In fact, it has been reported that the  $\delta^{18}\text{O}$  of  
619 water in developing grains exhibits a biphasic enrichment compared to stem water  
620 (Pande et al., 1994). The biphasic enrichment may be linked to developmental  
621 metabolism of the grain and rapid loss of water, together with oxidative metabolism  
622 during later stages of maturation (Pande et al., 1995). Such biphasic enrichment in the  
623 grains could therefore affect  $\delta^{18}\text{O}$ , which consequently might have enriched the  $\delta^{18}\text{O}$  of  
624 water from the developing grains compared to that in stem water (Table 2).  
625 Accordingly, the enrichment of water in the grain could be an additional factor that may  
626 hinder the registration of environmental conditions in the  $\delta^{18}\text{O}_{\text{grain}}$  dry matter. Moreover,  
627 the  $\delta^{18}\text{O}$  of water in the flag leaves was enriched compared to that in water from  
628 developing grains and stems (Table 2 and Fig. 2), which also agrees with the widely  
629 reported strong evaporation processes taking place in the leaf (Farquhar and Gan, 2003;  
630 Barbour et al., 2004). Besides, the  $\delta^{18}\text{O}$  from the water of photosynthetic and transpiring  
631 organs such as the flag leaves was strongly correlated with *GY* (Table 3) when all  
632 growing conditions were included. In short, the strong correlation between *GY* and  $\delta^{18}\text{O}$   
633 from water in leaves suggests that leaf water mainly reflects evaporative enrichment and  
634 thus environmental conditions, with the additional advantage (at least in the case of

635  $\delta^{18}\text{O}$ ) of avoiding the fractionation associated with biochemical reactions during the  
636 synthesis of organic matter (Farquhar and Lloyd, 1993).

637

638 *Applicability of plant  $\delta^2\text{H}$  to assess plant performance*

639 Strong correlations between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in the cellulose of leaves have been reported  
640 in the literature, which suggests similar sources of variation for plant isotopic signals  
641 (Epstein et al., 1977), whereas the absence of a correlation would indicate additional  
642 biochemical effects (Sternberg et al., 1986). Keeping this in mind, in the 2010 season  
643 the absence of significant correlations between  $\delta^{18}\text{O}_{\text{grain}}$  and  $\delta^2\text{H}_{\text{grain}}$  (Fig. 3), together  
644 with the lack of any relationship between  $\delta^{18}\text{O}_{\text{grain}}$  and *GY*, suggests that  $\delta^{18}\text{O}_{\text{grain}}$  is  
645 more sensitive to biochemical reactions than  $\delta^2\text{H}_{\text{grain}}$  during grain formation (Farquhar  
646 and Lloyd, 1993) or is more likely to undergo exchange with the ( $^{18}\text{O}$  of) source water  
647 (Barbour, 2007).

648

649 Although exchange of hydrogen isotopes with water within the cytosol can affect the  
650  $\delta^2\text{H}$  of organic compounds and the contribution to NADPH (Zhou et al., 2018), the  
651 analysis of  $\delta^2\text{H}$  depletion in plant organs could be used as an indicator of the net  
652 isotopic effect associated with NADPH synthesis in the chloroplast (Hayes, 2001; Zhou  
653 et al., 2010). Therefore, if NADPH is not regenerated continuously, reduction power  
654 can be strictly limited and distinct metabolic processes such as photosynthetic electron  
655 transport or nitrate reduction (Bloom, 2015) may inhibit carbon fixation (Foyer et al.,  
656 2012). In agreement with our results (Fig. 3), data from a study in mature kernels of  
657 wheat (Liu et al., 2015) also showed a strong positive correlation between  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$ ,  
658 supporting the idea that both isotopes composition (even in heterotrophic organs) are  
659 affected by photosynthetic activity even if the source of variation is different. Thus, for  
660 example, whereas a decrease in photosynthetic activity caused by water stress is the  
661 consequence of a lower  $\text{CO}_2$  availability (which increases  $\delta^{13}\text{C}$ ) it diminishes the  
662 synthesis of NADPH produced in the chloroplast (and then  $\delta^2\text{H}$  decreases less).

663

664 However, as discussed above for  $\delta^{18}\text{O}$ , the  $\delta^2\text{H}$  of organic matter may also be affected  
665 by the source water  $\delta^2\text{H}$  (Fig. 2), and subjected to evaporative enrichment in the leaves  
666 and to biphasic enrichment in grains. Nevertheless, in spite of these fractionation  
667 processes, there were good correlations of the  $\delta^2\text{H}$  from the flag leaves, ears and grains

668 with  $g_s$  and  $GY$  (Fig. 4). In fact, the only isotope composition that correlated with  $GY$  (as  
669 a dependent variable) in the stepwise analysis in 2010 was the  $\delta^2H$  under SI and RF  
670 conditions (SI  $r=0.75$   $P= 0.000$ ; RF  $r=0.62$   $P= 0.006$ ). In contrast to  $\delta^{18}O$ , these results  
671 suggest that  $\delta^2H$  was not hindered by fractionation processes within different organs,  
672 during transport of assimilates to the grains or during heterotrophic metabolism within  
673 the grains. Therefore,  $\delta^2H$  may provide simultaneous time-integrated records of the  
674 photosynthetic and evaporative performance of the plant during crop development,  
675 based on, among other aspects, its tighter association with  $\delta^{13}C$  than with  $\delta^{18}O$ .

676

677

## 678 **CONCLUSIONS**

679 In autotrophic organs, such as the flag leaf,  $\delta^2H$  was not only affected by changes in  
680 transpiration and stomatal conductance but also by photosynthetic carbon metabolism  
681 because the net isotopic effect ( $\delta^2H$  depletion) was negatively associated with ETR.  
682 Contrastingly,  $\delta^2H$  enrichment in heterotrophic organs such as the grains and roots was  
683 associated with post-photosynthetic effects as there are several processes that lead to  
684  $^2H$ -enrichment of carbohydrates. In the case of the ears, their intermediate  $\delta^2H$  values  
685 (lying between the flag leaves and grains) may be the consequence of different factors  
686 such as lower transpiration compared to that in the leaves, the mixotrophic nature of the  
687 bracts or some degree of CAM metabolism.

688 The significant correlations between  $\delta^2H$  and grain yield and the existence of genotypic  
689 variability in plant  $\delta^2H$  are encouraging when considering this isotope for assessing  
690 plant performance under different growing conditions.

691

692

693

## 694 **MATERIALS AND METHODS**

695

### 696 *Germplasm used and experimental conditions*

697 Ten durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) genotypes were  
698 sown: five historical Spanish landraces (*Blanqueta*, *Griego de Baleares*, *Negro*, *Jerez*

699 37 and *Forment de Artes*) and five modern Spanish commercial varieties released after  
700 1990 (*Anton, Bolo, Don Pedro, Regallo* and *Sula*). Landraces were chosen based on  
701 their similarity to the phenology of modern cultivars. Field experiments were conducted  
702 during the 2010 and 2011 growing seasons at the experimental station of the Instituto  
703 Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) of Aranjuez  
704 (40°03'N, 3°31'E, 500 m asl) with experimental conditions explained elsewhere  
705 (Sanchez-Bragado et al., 2014a). Two water treatments (support irrigation, SI, and rain-  
706 fed, RF) combined with two nitrogen regimes (fertilized, HN, and non-fertilized, LN)  
707 were assayed. The trials were planted on 30 December 2010 and 18 November 2011 in  
708 plots with six rows 0.20 m apart, covering a total area of 6 m<sup>2</sup> (5 m length and 1.2 m  
709 width) per plot. Total accumulated precipitation during the 2010 and 2011 seasons was  
710 275.4 and 126.1 mm, respectively. For both years, sprinkler irrigation was applied to  
711 irrigated plots around GS41 (Zadoks et al., 1974) (beginning of April) and GS71  
712 (around May 15<sup>th</sup> and 30<sup>th</sup>) with approximately 60 mm supplied on each date.  
713 Environmental conditions during growth are detailed in Fig. 5. Prior to sowing, all trials  
714 received 60 kg ha<sup>-1</sup> of phosphorous as superphosphate (18%) and 60 kg ha<sup>-1</sup> potassium  
715 as potassium chloride (60%). Further, the HN plants were dressed with nitrogen applied  
716 at the beginning of tillering (January 27<sup>th</sup> in 2010 and December 29<sup>th</sup> in 2011) and  
717 jointing (March 20<sup>th</sup> in 2010 and February 20<sup>th</sup> in 2011) using a dose of 45 kg ha<sup>-1</sup> and  
718 105 kg ha<sup>-1</sup> of urea (46%), respectively. The LN plants were not N fertilized, relying  
719 exclusively on the N available in the soil before sowing. Water and nitrogen treatments  
720 were arranged according to a split-split plot design with three replicates. Experimental  
721 plots were kept free of weeds, insects, pests and diseases by recommended chemical  
722 measures (Sanchez-Bragado et al., 2014a).

723

724 Sampling was performed around 7 days after anthesis, corresponding to the GS71-75  
725 Zadocks stages (Zadoks et al., 1974) in 2010 and two weeks after anthesis (stages  
726 GS75-81) in 2011. In 2010 the genotype *Foment de Artes* was discarded due to late  
727 phenology. Also, in 2011 all five landraces under support irrigation were discarded due  
728 to lodging. In 2010 roots were collected from the upper layer (0–10cm) with a split tube  
729 sampler (Ref. 04.17.01.C, Eijkelkamp Soil & Water, The Netherlands), rinsed with  
730 distilled water and then placed inside a paper envelope. Thereafter, five representative  
731 flag leaves and ears were collected per plot, and oven dried together with collected roots  
732 at 70°C for 48 h, then weighed and finely ground for hydrogen, oxygen and carbon

733 isotope analyses (in total dry matter). In 2011, flag leaves and developing grains from  
734 five representative tillers were collected and immediately frozen for subsequent water  
735 extraction (see below). Stomatal conductance ( $g_s$ ) was measured with a leaf porometer  
736 (Decagon; <http://www.decagon.com>) in one leaf per plot. At maturity, the central four  
737 rows of each plot were harvested and grain yield ( $GY$ ) recorded. Subsequently, mature  
738 kernels were processed as explained below for isotope analysis. Harvest was performed  
739 manually and by machine in 2010 and 2011, respectively.

740

#### 741 *Carbon isotope analyses*

742 Carbon isotope analyses of mature grains as well as the total dry matter (DM) and  
743 water-soluble fraction (WSF) of the flag leaf blades and ears from the field trials,  
744 together with the DM of the flag leaves from the growth chamber experiment, were  
745 performed using an Elemental Analyzer (Flash 1112 EA; ThermoFinnigan, Bremen,  
746 Germany) coupled with an Isotope Ratio Mass Spectrometer (Delta C IRMS,  
747 ThermoFinnigan, Bremen, Germany) operating in continuous flow mode in order to  
748 determine the stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ) isotope ratios of the same samples. Samples of  
749 approximately 1 mg of total dry matter for mature grains, 0.7 mg for flag leaves and  
750 ears and reference materials were weighed into tin capsules, sealed, and then loaded into  
751 an automatic sampler (ThermoFinnigan, Bremen, Germany) before EA-IRMS analysis.  
752 The  $^{13}\text{C}/^{12}\text{C}$  ratios of plant material were expressed in  $\delta$  notation (Coplen, 2008):  
753  $\delta^{13}\text{C} = (^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}} - 1$ , where ‘sample’ refers to plant material  
754 and ‘standard’ to international secondary standards of known  $^{13}\text{C}/^{12}\text{C}$  ratios (IAEA CH7  
755 polyethylene foil, IAEA CH6 sucrose and USGS 40 l-glutamic acid) calibrated against  
756 Vienna Pee Dee Belemnite calcium carbonate with an analytical precision (standard  
757 deviation) of 0.10‰.

758

759 Measurements were carried out at the Scientific Facilities of the University of  
760 Barcelona. The  $\delta^{13}\text{C}$  of flag leaves (DM), ears (DM), roots and mature kernels are  
761 referred to as  $\delta^{13}\text{C}_{\text{flagDM}}$ ,  $\delta^{13}\text{C}_{\text{earDM}}$ ,  $\delta^{13}\text{C}_{\text{rootsDM}}$  and  $\delta^{13}\text{C}_{\text{grain}}$ , respectively.

762

#### 763 *Oxygen isotope analyses*

764 The  $^{18}\text{O}/^{16}\text{O}$  ratios of the same mature grains as well as the total DM and water-soluble  
765 fraction (WSF) of flag leaf blades and ears were determined by an on-line pyrolysis  
766 technique using a Thermo-Chemical Elemental Analyzer (TC/EA Thermo Quest

767 Finnigan, Bremen, Germany) coupled with an IRMS (Delta C Finnigan MAT, Bremen,  
768 Germany). Samples of 1 mg of total dry matter for mature grains, flag leaves, ears and  
769 roots and reference materials were weighed into silver capsules, sealed and oven-dried  
770 at 60°C for no less than 72 h to remove moisture and loaded into an automatic sampler.  
771 Results were expressed as  $\delta^{18}\text{O}$  values, using two secondary standards (IAEA 601 and  
772 IAEA 602) calibrated against Vienna Standard Mean Oceanic Water (VSMOW), and  
773 the analytical precision was  $\sim 0.25\%$ . Analyses were conducted at Iso-Analytical  
774 Limited (Crewe, Cheshire CW2 8UY, UK). The  $\delta^{18}\text{O}$  of flag leaves (DM), ears (DM),  
775 roots and mature kernels are referred to as  $\delta^{18}\text{O}_{\text{flagDM}}$ ,  $\delta^{18}\text{O}_{\text{earDM}}$ ,  $\delta^{18}\text{O}_{\text{rootsDM}}$  and  
776  $\delta^{18}\text{O}_{\text{grain}}$ , respectively.

777

#### 778 *Hydrogen isotope analyses*

779 The  $^2\text{H}/^1\text{H}$  ratios of the same mature grains as well as the total dry matter (DM) and  
780 water-soluble fraction (WSF) of the flag leaf blades and ears (and only leaves DM in the  
781 growing chamber experiment) were determined by an on-line pyrolysis technique using  
782 a Thermo-Chemical Elemental Analyzer (TC/EA Thermofisher Scientific Inc, Bremen,  
783 Germany) coupled with an IRMS (Delta plus xp, Bremen, Germany). Samples of 0.15  
784 mg of total dry matter for mature grains, flag leaves, ears, roots and reference materials  
785 were weighed into silver capsules, sealed and oven-dried at 60°C for not less than 72 h  
786 to remove moisture and then loaded into an automatic sampler. In addition, samples  
787 were always kept under free moisture conditions with silica gel in a desiccator. Results  
788 were expressed as  $\delta^2\text{H}$  values, using international secondary standards (for calibration  
789 and checking precision and accuracy) of known  $^2\text{H}/^1\text{H}$  ratios (IAEA CH7 polyethylene  
790 foil, 5 $\alpha$ -androstande, coumarin and eicosanoic acid methyl ester) calibrated against  
791 VSMOW, and the analytical precision was  $\sim 0.5\%$ . In addition, a secondary internal  
792 standard (IAEA 601,  $\delta^2\text{H}=-85.1\%$ ) was selected to provide at least a two-point  
793 calibration (normalization) of the hydrogen isotope delta scale anchored by VSMOW.  
794 Measurements were carried out at the Scientific Facilities of the University of  
795 Barcelona. The  $\delta^2\text{H}$  of flag leaves (DM), ears (DM), roots and mature kernels are  
796 referred to as  $\delta^2\text{H}_{\text{flagDM}}$ ,  $\delta^2\text{H}_{\text{earDM}}$ ,  $\delta^2\text{H}_{\text{rootsDM}}$  and  $\delta^2\text{H}_{\text{grain}}$ , respectively.

797

#### 798 *Water-soluble fraction*



799 The protein-free water-soluble fractions (WSFs) of the flag leaves and ears were  
800 extracted from the same dry samples tested for carbon, hydrogen and oxygen isotopes,  
801 as described previously (Cabrera-Bosquet et al., 2011; Yousfi et al., 2013). Aliquots of  
802 40  $\mu\text{l}$  (carbon), 20  $\mu\text{l}$  (hydrogen), and 100  $\mu\text{l}$  (oxygen) of supernatant containing  
803 protein-free WSF were transferred into tin capsules for carbon analysis and into silver  
804 capsules for hydrogen and oxygen analyses. The capsules containing the aliquots were  
805 oven dried at 60°C. The WSFs of the  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of flag leaves and ears are  
806 referred to as  $\delta^{13}\text{C}_{\text{flag}}\text{WSF}$ ,  $\delta^{13}\text{C}_{\text{ear}}\text{WSF}$ ,  $\delta^2\text{H}_{\text{flag}}\text{WSF}$ ,  $\delta^2\text{H}_{\text{ear}}\text{WSF}$ ,  $\delta^{18}\text{O}_{\text{flag}}\text{WSF}$  and  
807  $\delta^{18}\text{O}_{\text{ear}}\text{WSF}$ , respectively. Additionally, in order to estimate any possible exchange  
808 between the samples and the water used to extract the protein-free WSF, the powdered  
809 samples were suspended using three water reference sources with different  $\delta^2\text{H}$  (snow  
810 water,  $\delta^2\text{H}=-77.5\text{‰}$ ; deuterated water,  $\delta^2\text{H}=-94.4\text{‰}$  and seawater,  $\delta^2\text{H}=-3.3\text{‰}$ ). In fact,  
811 using extraction water sources with different  $\delta^2\text{H}$  signatures does not significantly affect  
812 the  $\delta^2\text{H}$  of the soluble fraction, and the absolute differences in  $\delta^2\text{H}$  between soluble  
813 fractions extracted from the dry matter with the different water sources were minor  
814 (Table S3).

815

#### 816 *Hydrogen and oxygen composition in plant water*

817 To determine source water variations in the 2010 and 2011 field experiments, a portion  
818 of the stem base was harvested in the field. In 2010, variations in source water were  
819 determined from pressed stem juice. Stem base segments were pressed with a high-  
820 pressure press in order to obtain a liquid extract. Subsequently, extracted liquid was  
821 transferred to 2-mL glass vials with crimp caps. Glass vials were sealed and sterilized in  
822 a water bath at 100°C for 2 h to prevent fermentation processes, and kept cool until  
823 isotope analysis. In 2011, a portion of the stem base was placed into sealed tubes  
824 immediately after sampling and subsequently frozen in a freezer at -20°C. Thereafter,  
825 water was extracted from the stem base using a cryogenic vacuum distillation line  
826 (Dawson and Ehleringer, 1993). The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of water extracted from the stem are  
827 referred to as  $\delta^2\text{H}_{\text{stemW}}$  and  $\delta^{18}\text{O}_{\text{stemW}}$ , respectively.

828

829 In 2011, flag leaves and developing grains were collected and placed into sealed tubes  
830 and frozen immediately after sampling. Thereafter, water was extracted from the  
831 developing grains and flag leaves using a cryogenic vacuum distillation line (Dawson

832 and Ehleringer, 1993) and measured together with stem water samples. The  $\delta^2\text{H}$  and  
833  $\delta^{18}\text{O}$  of water extracted from flag leaves and developing grains are referred to as  
834  $\delta^2\text{H}_{\text{flagW}}$ ,  $\delta^2\text{H}_{\text{grainW}}$ ,  $\delta^{18}\text{O}_{\text{flagW}}$  and  $\delta^{18}\text{O}_{\text{grainW}}$ , respectively.

835

836 Oxygen and hydrogen compositions ( $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$ ) in water distilled from stem bases, flag  
837 leaves and developing grains (experiment 2011) and stem juice extracts (stem water,  
838 experiment 2010) were determined by laser spectroscopy at the Serveis Científico-  
839 Tècnics of the Universitat de Lleida using a Picarro L2120i (Picarro Inc. California,  
840 USA) coupled to a high-precision vaporizer A0211. All samples were centrifuged at  
841 12,000 g in order to remove any suspended solid, and the supernatants transferred to  
842 glass vials with a 250-mL insert. In the case of stem juice, because large amounts of  
843 sugars reduce the performance of the vaporizer, juice samples were diluted to 50% with  
844 distilled water of known isotopic composition prior to injection as explained elsewhere  
845 (Sánchez-Bragado et al., 2016). At the same time, the potential presence of organic  
846 contaminants was checked using the post-processing software, Picarro ChemCorrect  
847 1.2.0 (Picarro Inc. California, USA) giving in some cases positive results.  
848 Consequently, the data was thereafter corrected for consistency across all samples to  
849 avoid undesired effects of organic contaminants as described by Martín-Gómez et al.  
850 (2015). Nevertheless, we found very strong correlations between corrected and  
851 uncorrected values ( $r^2 = 0.998$  for  $\delta^{18}\text{O}$ ;  $r^2 = 0.992$  for  $\delta^2\text{H}$ ,  $N = 106$ ), with 83% of the  
852 samples showing differences lower than 0.4‰ for  $\delta^{18}\text{O}$  and 4‰ for  $\delta^2\text{H}$ .

853

854 Isotopes were expressed in delta ( $\delta$ ) notation (‰) relative to V-SMOW (i.e. isotopic  
855 composition of oxygen,  $\delta^{18}\text{O}$ , and hydrogen,  $\delta^2\text{H}$ ). Raw values were calibrated against  
856 three internal laboratory references (calibrated against IAEA standards VSMOW2,  
857 SLAP2 and GISP). Overall uncertainty, determined as the standard error of repeated  
858 analyses, ( $N=20$ ) of a reference material not included in the calibration, was 0.05‰ and  
859 0.17‰, for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , respectively.

860

861

#### 862 *Dual-water equilibration method to quantify the fraction of exchangeable H*

863 A dual-water equilibration method was performed in order to quantify the fraction of  
864 exchangeable H and to determine the  $\delta^2\text{H}$  in the non-exchangeable H fraction

865 (Schimmelmann et al., 2001; Sauer et al., 2009; Qi and Coplen, 2011). Dry leaf material  
 866 and the water-soluble fraction of the same sample, plus standards, were used for the  
 867 dual-water equilibration method. A set of three aliquots extracted from the same sample  
 868 plus the standards were weighed and loaded into individual silver capsules, and each  
 869 capsule was held in a plastic tray and each plastic tray was placed in a glass desiccator  
 870 for equilibration with water sources with different  $\delta^2\text{H}$ . One set was equilibrated in a  
 871 glass desiccator with water depleted in  $^2\text{H}$  (ambient snow water,  $\delta^2\text{H}=-77.5\text{‰}$ ) and the  
 872 second set was equilibrated with seawater (ambient seawater,  $\delta^2\text{H}=-3.3\text{‰}$ ). In each  
 873 glass desiccator a set of standards were also included. Samples were equilibrated in each  
 874 desiccator for 7 days at ambient temperature ( $25^\circ\text{C}$ ). In order to remove the moisture  
 875 prior the equilibration period, the desiccators were purged with helium for five minutes  
 876 at  $120\text{ mL min}^{-1}$ . Subsequently, samples from light and heavy water were dried in  
 877 separate desiccators filled with Sicapent<sup>®</sup> ( $\text{P}_2\text{O}_5$ ) for at least 7 days. In parallel, a set of  
 878 identical samples used for water equilibration was oven dried at  $50^\circ\text{C}$  for 7 days. After  
 879 7 days of oven and Sicapent drying,  $\delta^2\text{H}$  measurements were performed with an on-line  
 880 pyrolysis technique using a Thermo-Chemical Elemental Analyzer (TC/EA  
 881 Thermofisher Scientific Inc, Bremen, Germany) coupled with an IRMS (Delta plus xp,  
 882 Bremen, Germany) as described above. In addition, the samples in the TC/EA carousel  
 883 were purged with helium ( $120\text{ mL min}^{-1}$ ). The helium purged gas was fed from the top  
 884 of the TC/EA reactor. All results were normalized to the VSMOW- SLAP isotope scale  
 885 (Coplen, 1988), using LIMS (Laboratory Information Management System) for Light  
 886 Stable Isotope so that the  $\delta^2\text{H}_{\text{VSMOW}}$  of SLAP (Standard Light Antarctic  
 887 Precipitation) was  $-428\text{‰}$  relative to VSMOW (Gonfiantini, 1978). Results were  
 888 expressed as  $\delta^2\text{H}$  values, using the same international secondary standards of known  
 889  $^2\text{H}/^1\text{H}$  ratios indicated before and the analytical precision was  $\sim 0.5\text{‰}$ . Finally, the  
 890 fraction of total hydrogen that is exchangeable ( $f_e$ ) was calculated as described by  
 891 Schimmelmann et al. (2001):

$$892 \quad f_e = (\delta^2 H_{TA} - \delta^2 H_{TB}) / (\delta^2 H_{WA} - \delta^2 H_{WB}) \quad (1)$$

893 where  $\delta^2 H_{TA}$  and  $\delta^2 H_{TB}$  are the  $\delta^2\text{H}$  values of the total hydrogen of the same samples  
 894 equilibrated with water standards  $wA$  and  $wB$ , respectively, and  $\delta^2 H_{WA}$  and  $\delta^2 H_{WB}$  are the  
 895  $\delta^2\text{H}$  values of water vapor A and B, respectively. Then the isotopic composition of non-  
 896 exchangeable hydrogens ( $\delta^2 H_n$ ) can be calculated as follows:

897 
$$\delta^2H_n = (\delta^2H_{TA} - f_e(\delta^2H_{WA} - \epsilon))/(1 - f_e) \quad (2)$$

898 where  $\epsilon$  is the fractionation effect between exchangeable organic hydrogen and water  
899 hydrogen. Although  $\epsilon$  might range from 30% to 110%, depending on the molecule  
900 structure containing organic exchangeable H (Schimmelmann, 1991), typical values for  
901 most material of interest in environmental studies (proteins, cellulose, humic acids)  
902 presented a fractionation effect of 80‰  $\pm$  20‰ (Wassenaar and Hobson, 2000).  
903 Therefore the  $\epsilon$  factor used in the calculation was 80‰ as has been previously  
904 determined experimentally for well-defined cellulose (Schimmelmann, 1991). Then the  
905 true  $\delta^2H_n$  values of the WSF and DM were calculated (Table S4) and the  $\delta^2H$  of the  
906 WSF and DM in the flag leaves, the ears and grains were corrected using the fraction of  
907 total hydrogen that is exchangeable obtained within the different equilibration  
908 conditions. Thus the  $\delta^2H$  of the WSF and DM presented in the results is the  $\delta^2H$  of non-  
909 exchangeable H of the organics. However, the dry matter fraction did not show any  
910 significant exchange with surrounding moisture (see Fig. S2). Dry organic material is  
911 likely to be in a temporary hydrophobic state and thus the exchange is much slower than  
912 in the hydrated form (Schimmelmann A., pers. com.; Wassenaar and Hobson, 2000).  
913 Moreover, leaf samples were exposed to the same kind of atmospheric moisture, so the  
914 exchangeable hydrogen was equilibrated to a common background of water. This  
915 ensures that relative differences among samples in the dry matter can be interpreted in  
916 terms of environmental (i.e. growing conditions) and biological (i.e. biochemical  
917 composition of leaf material) parameters. Nevertheless, the  $\delta^2H$  values of the DM  
918 presented in the results were also corrected using the fraction of total hydrogen that is  
919 exchangeable obtained within the different equilibration conditions.

920

921 In addition, to prove that the extraction water had not influenced the  $\delta^2H$  of the WSF,  
922 further extractions were performed on aliquots of DM from representative samples (the  
923 same samples used in the dual-water equilibration method) using four sources of water  
924 with different  $\delta^2H$ : snow water (-77.5‰), deuterated water (+94.4‰), seawater (-3.3‰)  
925 and lab water (-43.2‰). Furthermore, the differences between the WSFs were  
926 compared. However, using water sources with different  $\delta^2H$  signatures for extraction  
927 did not significantly affect the  $\delta^2H$  of the soluble fraction and the absolute differences in  
928  $\delta^2H$  between soluble fractions extracted from the DM with the different water sources

929 were minor (Table S4). All the measurements were carried out at the Scientific  
930 Facilities of the University of Barcelona.

931

#### 932 *Experimental estimation of the ETR's association with $\delta^2\text{H}$ depletion*

933 A modern Spanish durum wheat cultivar (Sula) was grown in 3-L pots (three replicates)  
934 filled with sand (one plant per pot). Plants were watered three times a week with  
935 Hoagland nutrient solution and were grown under controlled conditions in a growth  
936 chamber (Convicon E15, Controlled Environments Ltd., Winnipeg, Canada). Plants  
937 were supplied with a PPFD of about  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  at plant level during the light  
938 period (14 h). Plants were grown in a constant relative humidity of 40% or 80% within  
939 two different growth chambers with a temperature of 23/17°C during the light and dark  
940 periods, respectively. Six flag leaves from the main tiller of different plants grown  
941 under different relative humidity (40% or 80%) and the electron transport rate (ETR),  
942 stomatal conductance ( $g_s$ ) and photosynthetic rate of the flag leaf blades were measured  
943 using a LI-6400XT portable gas exchange photosynthesis system (Li-COR, Lincoln,  
944 Nebraska, USA), approximately two weeks after anthesis. The ETR,  $g_s$ , and  
945 photosynthetic rate were estimated at a saturating PPFD of  $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$  and 20°C.

946 Following the ETR measurements, the same six flag leaves from each of the RH  
947 conditions the  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  in the DM of the leaves were then analyzed as previously  
948 mentioned (see the hydrogen and carbon isotope analysis section).

949

950 In order to support a causal association between the ETR and the effect that  $^2\text{H}$ -depleted  
951 NADPH has on  $\delta^2\text{H}$  of the organic matter, the  $\delta^2\text{H}$  and the ETR in the flag leaf were  
952 compared between the different RH treatments (Table S1).

953

#### 954 *Statistical analysis*

955 Grain yield, agronomic components and isotopic data were subjected to one way  
956 analyses of variance (ANOVA) using the general linear model to calculate the effects of  
957 water regime, nitrogen supply, genotype and their interactions with the studied  
958 parameters. Water regime, nitrogen supply, and genotype were included as fixed factors  
959 including three blocks and three replicates per block. Means were compared by Tukey's  
960 HSD test and were performed on a combination of water treatments and nitrogen  
961 supply. Mean values across plant tissues with different letters (a, b, c and d) presented

962 in the tables are significantly different from SI vs. RF and HN vs. LN according to the  
963 Tukey's honestly significant difference test ( $P < 0.05$ ). A bivariate correlation procedure  
964 was constructed to analyze the relationships between the measured traits. Statistical  
965 analyses were performed using the SPSS 18.0 statistical package (SPSS Inc., Chicago,  
966 IL, USA). Figures were created using the Sigma-Plot 10.0 program (SPSS Inc.).  
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968

## 969 SUPPLEMENTAL DATA

970 Is composed of:

971 **Supplemental Figure S1.** Polynomial regression between the mean grain yield of the  
972 trials and the regression coefficient of the relationship between grain yield carbon,  
973 oxygen and hydrogen isotope compositions of different plant components.

974

975 **Supplemental Figure S2.** Mean values of stable isotope composition (‰) of hydrogen  
976 ( $\delta^2\text{H}$ ) in the water-soluble fraction (WSF) and dry matter (DM) obtained in the dual-  
977 water equilibration method.

978

979 **Supplemental Table S1.** Mean values of isotope composition (‰) of hydrogen( $\delta^2\text{H}$ )  
980 and carbon ( $\delta^{13}\text{C}$ ) in dry matter and the ETR,  $g_s$  and photosynthetic rate of the flag leaf  
981 of plants grown under two different relative humidity conditions in the growth chamber  
982 experiment.

983

984 **Supplemental Table S2.** Linear regression of the relationship of the carbon ( $\delta^{13}\text{C}$ )  
985 oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) isotope compositions in the water-soluble fraction  
986 (WSF) and dry matter (DM) of the flag leave, ears and mature grains with the grain  
987 yield ( $GY$ ).

988

989 **Supplemental Table S3.** Mean values of stable isotope composition (‰) of hydrogen  
990 ( $\delta^2\text{H}$ ) in the water-soluble fraction (WSF) using four sources of water with different  $\delta^2\text{H}$   
991 for the extraction of the WSF.

992

993 **Supplemental Table S4.** Mean values of stable isotope composition (‰) of hydrogen  
994 ( $\delta^2\text{H}$ ) in the flag leaf water-soluble fraction (WSF) and dry matter (DM) obtained with  
995 the dual water equilibration method.

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1010 **TABLES**

1011

1012 **Table 1.** Mean values of grain yield (*GY*), stomatal conductance ( $g_s$ ) and stable isotope  
1013 composition (‰) of hydrogen ( $\delta^2\text{H}$ ), oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) of dry matter  
1014 (DM) and the water-soluble fraction (WSF) of different plant parts (flag leaves, ears and  
1015 roots) sampled at mid grain filling plus mature kernels (grains) and in the water from  
1016 the basal part of the stem (stem water) under support irrigation (SI), rainfed (RF),  
1017 nitrogen fertilized (HN) and non-fertilized (LN) conditions, as examined in modern  
1018 cultivars (cultivars) and old landraces (landraces).  $\delta^{13}\text{C}$  DM and  $\delta^{13}\text{C}$  WSF, and  $\delta^{18}\text{O}$   
1019 WSF and  $\delta^2\text{H}$  WSF were measured in 108 plots (five cultivars and four landraces, four  
1020 growing conditions and three replicates), whereas  $\delta^{18}\text{O}$  DM and  $\delta^2\text{H}$  DM were  
1021 measured in 48 plots (two cultivars and two landraces, four growing conditions and  
1022 three replicates) during the 2010 crop cycle. Each value represents the mean  $\pm$  SD.  
1023 Mean values across plant tissues with different letters are significantly different from SI  
1024 vs. RF and HN vs. LN and landraces vs. cultivars according to the Tukey's honestly  
1025 significant difference test ( $P < 0.05$ ).

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Isotope/organ/fraction	Cultivars	Landraces	SI	RF	HN	LN
<b>Hydrogen</b>						
$\delta^2\text{H}_{\text{roots}}\text{DM}$	-67.1 ± 18.6a	-73.2 ± 20.6a	-64.3 ± 23.5a	-75.8 ± 13.3b	-72.5 ± 17.7a	-68.0 ± 21.5a
$\delta^2\text{H}_{\text{stemW}}$	-46.4 ± 7.2a	-45.3 ± 5.7a	-46.3 ± 7.6a	-45.6 ± 5.6a	-47.1 ± 7.3a	-44.8 ± 5.7a
$\delta^2\text{H}_{\text{flag}}\text{DM}$	-114.9 ± 8.7a	-115.5 ± 8.8a	-120.6 ± 6.7a	-109.6 ± 6.8b	-111.5 ± 9.6a	-119.1 ± 5.5b
$\delta^2\text{H}_{\text{flag}}\text{WSF}$	-100.9 ± 6.9a	-104.4 ± 7.4b	-104.1 ± 7.8a	-100.9 ± 6.4b	-101.8 ± 8.1a	-103.2 ± 6.4a
$\delta^2\text{H}_{\text{ear}}\text{DM}$	-90.0 ± 8.6a	-95.3 ± 10.6a	-99.0 ± 7.1a	-86.4 ± 8.1b	-88.9 ± 10.0a	-96.4 ± 8.4b
$\delta^2\text{H}_{\text{ear}}\text{WSF}$	-65.7 ± 7.0a	-71.4 ± 9.2b	-72.5 ± 6.7a	-64.3 ± 8.0b	-65.4 ± 9.5a	-71.2 ± 6.4b
$\delta^2\text{H}_{\text{grain}}$	-33.6 ± 8.2a	-30.9 ± 9.4a	-36.6 ± 6.6a	-28.2 ± 8.9b	-27.4 ± 7.7a	-35.8 ± 6.9b
<b>Oxygen</b>						
$\delta^{18}\text{O}_{\text{roots}}\text{DM}$	28.1 ± 7.5a	30.8 ± 8.8a	29.6 ± 9.0a	29.3 ± 7.6a	32.3 ± 9.4a	26.7 ± 5.9b
$\delta^{18}\text{O}_{\text{stemW}}$	-6.3 ± 1.0a	-6.1 ± 0.6a	-6.4 ± 0.9a	-6.0 ± 0.8a	-6.4 ± 0.9a	-6.0 ± 0.7a
$\delta^{18}\text{O}_{\text{flag}}\text{DM}$	30.8 ± 1.7a	31 ± 1.1a	30.3 ± 1.0a	31.4 ± 1.6b	31.1 ± 1.6a	30.7 ± 1.3a
$\delta^{18}\text{O}_{\text{flag}}\text{WSF}$	30.7 ± 2.2a	30 ± 2.3a	28.9 ± 1.9a	32.3 ± 0.9b	29.6 ± 2.7a	31.2 ± 1.3a
$\delta^{18}\text{O}_{\text{ear}}\text{DM}$	26.6 ± 2.4a	26.1 ± 2.6a	26.5 ± 2.0a	26.2 ± 2.9a	26.7 ± 2.2a	26.0 ± 2.7a
$\delta^{18}\text{O}_{\text{ear}}\text{WSF}$	30.9 ± 1.1a	29.9 ± 1.0a	30.2 ± 0.9a	30.7 ± 1.3a	30.3 ± 1.2a	30.7 ± 1.1a
$\delta^{18}\text{O}_{\text{grain}}$	30.4 ± 0.7a	30.6 ± 0.9b	30.4 ± 0.7a	30.6 ± 0.9a	30.3 ± 0.6a	30.7 ± 0.9a
<b>Carbon</b>						
$\delta^{13}\text{C}_{\text{flag}}\text{DM}$	-25.9 ± 0.9a	-25.5 ± 0.9b	-26.2 ± 0.7a	-25.2 ± 0.8b	-25.3 ± 0.8a	-26.2 ± 0.8b
$\delta^{13}\text{C}_{\text{flag}}\text{WSF}$	-27 ± 1.1a	-27.2 ± 1.1a	-27.9 ± 0.9a	-26.3 ± 0.7b	-27.2 ± 1.4a	-26.9 ± 0.7a
$\delta^{13}\text{C}_{\text{ear}}\text{DM}$	-24.5 ± 1.0a	-25 ± 0.9b	-25.4 ± 0.7a	-24.1 ± 0.8b	-24.5 ± 1.0a	-25.0 ± 0.9b
$\delta^{13}\text{C}_{\text{ear}}\text{WSF}$	-23.3 ± 0.9a	-24.4 ± 1.0b	-24.5 ± 0.9a	-23.1 ± 0.8b	-23.6 ± 1.1a	-24.0 ± 1.1a
$\delta^{13}\text{C}_{\text{grain}}$	-24.6 ± 0.9a	-23.9 ± 1.0b	-25.0 ± 0.6a	-23.5 ± 0.7b	-24.0 ± 1.1a	-24.6 ± 0.8b
$g_s$ (mmol·H <sub>2</sub> O·m <sup>-2</sup> ·s <sup>-1</sup> )	184.7 ± 78.1a	170.2 ± 59.0a	222.8 ± 56.2a	133.8 ± 52.8b	163.6 ± 84.5a	193.1 ± 49.0b
$GY$ (Mg·ha <sup>-1</sup> )	1.9 ± 0.1 a	1.5 ± 0.1 b	2.1 ± 0.1 a	1.3 ± 0.1 b	1.8 ± 0.1 a	1.6 ± 0.1 a

1040 **Table 2.** Mean values of grain yield (*GY*), stomatal conductance ( $g_s$ ) and carbon, oxygen  
1041 and hydrogen stable isotope compositions. Hydrogen isotope composition (‰) was  
1042 analyzed in the flag leaf water ( $\delta^2\text{H}_{\text{flagW}}$ ), grain water ( $\delta^2\text{H}_{\text{grainW}}$ ), stem water ( $\delta^2\text{H}_{\text{stemW}}$ )  
1043 and irrigation water ( $\delta^2\text{H}_{\text{sourceW}}$ ) in SI plots. Oxygen isotope composition (‰) was  
1044 analyzed in the flag leaf water ( $\delta^{18}\text{O}_{\text{flagW}}$ ), grain water ( $\delta^{18}\text{O}_{\text{grainW}}$ ), stem water  
1045 ( $\delta^{18}\text{O}_{\text{stemW}}$ ), irrigation water in SI ( $\delta^{18}\text{O}_{\text{sourceW}}$ ), and the dry matter (DM) of the flag leaves  
1046 ( $\delta^{18}\text{O}_{\text{flagDM}}$ ) and mature kernels ( $\delta^{13}\text{C}_{\text{grain}}$ ). Carbon isotope composition (‰) was  
1047 analyzed in the dry matter in the flag leaves ( $\delta^{13}\text{C}_{\text{flagDM}}$ ) and mature kernels ( $\delta^{13}\text{C}_{\text{grain}}$ ).  
1048  $\delta^{13}\text{C}$  analysis was performed on nine durum wheat genotypes and three replicates grown  
1049 under two different water conditions (support irrigation, SI vs. rain-fed, RF, including all  
1050 levels of nitrogen) accounting for a total of 54 plots. For water extracted from flag leaves,  
1051  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  were measured in a subset of 2 cultivars and 2 landraces (with three  
1052 replicates) under fertilized conditions and two water regimes (18 plots).  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$   
1053 were measured in extracted water from stems, developing grains and dry matter in a  
1054 subset of 5 cultivars and 5 landraces (with three replicates) under fertilized conditions and  
1055 two water regimes (45 plots) during the 2011 crop cycle (landraces were discarded due to  
1056 lodging under SI conditions, see Materials and Methods). Each value represents the mean  
1057  $\pm$  SD. Mean values across plant tissues with different letters are significantly different  
1058 according to the Tukey's honestly significant difference test ( $P < 0.05$ ).

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Isotope/water organ	SI	RF
<b>Hydrogen</b>		
$\delta^2\text{H}_{\text{flagW}}$	-8.1 $\pm$ 0.4a	17.9 $\pm$ 2.4b
$\delta^2\text{H}_{\text{grainW}}$	-26.2 $\pm$ 0.9a	-9.7 $\pm$ 2.3b
$\delta^2\text{H}_{\text{stemW}}$	-43.0 $\pm$ 0.6a	-43.8 $\pm$ 0.6a
$\delta^2\text{H}_{\text{sourceW}}$	-45.0	
<b>Oxygen</b>		
$\delta^{18}\text{O}_{\text{flagW}}$	3.0 $\pm$ 0.5a	15.5 $\pm$ 1.0b
$\delta^{18}\text{O}_{\text{grainW}}$	2.8 $\pm$ 0.2a	9.3 $\pm$ 0.7b
$\delta^{18}\text{O}_{\text{stemW}}$	-5.6 $\pm$ 0.1a	-5.6 $\pm$ 0.1a
$\delta^{18}\text{O}_{\text{sourceW}}$	-6.1	
$\delta^{18}\text{O}_{\text{flagDM}}$	27.7 $\pm$ 0.1a	33.3 $\pm$ 0.2b
$\delta^{18}\text{O}_{\text{grain}}$	30.2 $\pm$ 0.1a	32.3 $\pm$ 0.1b
<b>Carbon</b>		
$\delta^{13}\text{C}_{\text{flagDM}}$	-28.8 $\pm$ 0.1a	-26.5 $\pm$ 0.1b
$\delta^{13}\text{C}_{\text{grain}}$	-26.3 $\pm$ 0.1a	-24.4 $\pm$ 0.1b
$g_s$ (mmol·H <sub>2</sub> O·m <sup>-2</sup> s <sup>-1</sup> )	110.0 $\pm$ 36.3	29.1 $\pm$ 22.5
$GY$ (Mg·ha <sup>-1</sup> )	4.5 $\pm$ 0.1a	1.7 $\pm$ 0.1b

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1071 **Table 3.** Linear regression of the relationship between hydrogen isotope composition of  
1072 the flag leaf water ( $\delta^2\text{H}_{\text{flagW}}$ ), grain water ( $\delta^2\text{H}_{\text{grainW}}$ ) and stem water ( $\delta^2\text{H}_{\text{stemW}}$ ); oxygen  
1073 isotope composition of the flag leaf water ( $\delta^{18}\text{O}_{\text{flagW}}$ ), grain water ( $\delta^{18}\text{O}_{\text{grainW}}$ ), stem water  
1074 ( $\delta^{18}\text{O}_{\text{stemW}}$ ), and the dry matter of the flag leaves ( $\delta^{18}\text{O}_{\text{flagDM}}$ ), mature kernels ( $\delta^{18}\text{O}_{\text{grain}}$ )  
1075 and grain yield (GY). Carbon isotope composition of the dry matter in the flag leaves  
1076 ( $\delta^{13}\text{C}_{\text{flagDM}}$ ), mature kernels ( $\delta^{13}\text{C}_{\text{grain}}$ ) and grain yield (GY). The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of the  
1077 water extracted from the flag leaves were analyzed in a subset of 2 cultivars and 2  
1078 landraces (with three replicates) under fertilized conditions and two water regimes (18  
1079 plots) (landraces in SI conditions were discarded due to lodging). The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  were  
1080 measured in water from the stems, developing grains and dry matter in a subset of 5  
1081 cultivars and 5 landraces (with three replicates) under fertilized conditions and two water  
1082 regimes (45 plots) (landraces were discarded due to lodging under SI conditions, see  
1083 Materials and Methods). Analyses were performed in samples from the 2011 crop season.  
1084 Level of significance: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$  and ns, not significant.

Isotope/organ/fraction	$\delta^2\text{H}_{\text{flagW}}$	$\delta^2\text{H}_{\text{grainW}}$	$\delta^2\text{H}_{\text{stemW}}$	$\delta^{18}\text{O}_{\text{flagW}}$	$\delta^{18}\text{O}_{\text{grainW}}$	$\delta^{18}\text{O}_{\text{stemW}}$	GY
<b>Hydrogen</b>							
$\delta^2\text{H}_{\text{flagW}}$		0.66**	-0.13ns	0.99***	0.70**	0.01ns	-0.78***
$\delta^2\text{H}_{\text{grainW}}$	0.65**		-0.26ns	0.61**	0.99***	-0.21ns	-0.54***
$\delta^2\text{H}_{\text{stemW}}$	-0.13ns	-0.26ns		-0.12ns	-0.26ns	0.81***	0.12ns
<b>Oxygen</b>							
$\delta^{18}\text{O}_{\text{flagW}}$	0.99***	0.61**	-0.12ns		0.67**	0.04ns	-0.82***
$\delta^{18}\text{O}_{\text{grainW}}$	0.70**	0.99***	0.27ns	0.67**		-0.20ns	-0.62***
$\delta^{18}\text{O}_{\text{stemW}}$	-0.00ns	-0.21ns	-0.81***	0.04ns	-0.20ns		-0.07ns
$\delta^{18}\text{O}_{\text{flagDM}}$	0.96***	0.72***	-0.31ns	0.97***	0.82***	0.11ns	-0.93***
$\delta^{18}\text{O}_{\text{grain}}$	0.90***	0.73***	-0.43*	0.90***	0.80***	0.07ns	-0.83***
<b>Carbon</b>							
$\delta^{13}\text{C}_{\text{flagDM}}$	0.89***	0.69***	-0.28ns	0.92***	0.78***	0.16ns	-0.86***
$\delta^{13}\text{C}_{\text{grain}}$	0.65**	0.49**	-0.08ns	0.71**	0.59***	0.17ns	-0.93***

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1090 **Table 4.** Linear regression of the relationship of the carbon ( $\delta^{13}\text{C}$ ) oxygen ( $\delta^{18}\text{O}$ ) and  
1091 hydrogen ( $\delta^2\text{H}$ ) isotope compositions in the water-soluble fraction (WSF) and dry matter  
1092 (DM) of the flag leaves, ears and mature kernels (grains) with the grain yield ( $GY$ ),  
1093 stomatal conductance ( $g_s$ ), and total nitrogen concentration of the flag leaves (N-Flag)  
1094 and ears (N-Ear). Nine genotypes and three replicates per genotype were considered,  
1095 accounting for a total of 54 values under rainfed (water stress) conditions (RF) including  
1096 fertilized and non-fertilized conditions and under fertilized conditions (HN) including the  
1097 two water conditions. For the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of dry matter (flag leaves, ears and roots)  
1098 only two cultivars and two landraces were considered (24 plots). Analyses were  
1099 performed on samples from the 2010 crop season. Level of significance: \*\*\*,  $P < 0.001$ ;  
1100 \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; not significant, ns,  $P > 0.05$ .

Isotope/organ /fraction	g <sub>s</sub>				GY				N-Flag				N-Ear			
	SI	RF	HN	LN	SI	RF	HN	LN	SI	RF	HN	LN	SI	RF	HN	LN
<b>Hydrogen</b>																
$\delta^2\text{H}_{\text{roots}}$ DM	0.2ns	0.2ns	0.4ns	0.4ns	-0.2ns	-0.1ns	0.2ns	0.4ns	-0.1ns	-0.1ns	0.5*	-0.1ns	-0.2ns	-0.2ns	0.5*	-0.1ns
$\delta^2\text{H}_{\text{stem water}}$	-0.3*	0.4**	-0.1ns	0.1ns	-0.3ns	-0.1ns	-0.2ns	0.1ns	-0.1*	0.3*	-0.2ns	-0.1ns	0.2ns	0.1ns	0.3*	-0.0ns
$\delta^2\text{H}_{\text{flag}}$ DM	-0.2ns	-0.7***	-0.8***	-0.3ns	0.1ns	-0.5*	-0.6**	-0.3ns	0.3ns	0.5*	-0.6**	0.0ns	0.3ns	0.7***	-0.1ns	0.1ns
$\delta^2\text{H}_{\text{flag}}$ WSF	-0.2*	-0.6**	-0.4**	-0.1ns	-0.1ns	-0.3*	-0.4**	-0.1ns	-0.1*	0.6**	-0.4**	0.2ns	0.1ns	0.7***	0.2ns	0.2ns
$\delta^2\text{H}_{\text{ear}}$ DM	-0.2ns	-0.4**	-0.8***	-0.4ns	0.3ns	-0.4*	-0.6**	-0.4ns	0.5ns	0.4**	-0.3ns	0.4ns	0.5*	0.9***	0.2ns	0.5*
$\delta^2\text{H}_{\text{ear}}$ WSF	0.2ns	-0.6***	-0.4**	-0.3*	0.4**	-0.5***	-0.3*	-0.3*	0.4ns	0.7***	-0.1ns	0.1ns	0.3*	0.5***	0.1ns	0.1ns
$\delta^2\text{H}_{\text{grain}}$	0.1ns	-0.6***	-0.5***	-0.5***	0.3*	-0.4**	-0.5***	-0.5***	0.7ns	0.4**	-0.5***	0.1ns	0.6***	0.4**	-0.2ns	-0.0ns
<b>Oxygen</b>																
$\delta^{18}\text{O}_{\text{roots}}$ DM	0.1ns	-0.1ns	0.1ns	0.3ns	0.1ns	-0.2ns	0.1ns	0.3ns	0.5ns	-0.1ns	-0.1ns	-0.0ns	-0.3ns	0.1ns	-0.1ns	-0.0ns
$\delta^{18}\text{O}_{\text{xylem water}}$	0.3*	0.3*	0.3*	-0.0ns	0.3ns	-0.2ns	-0.3*	0.1ns	-0.2*	-0.3*	-0.2ns	-0.1ns	0.2ns	0.2ns	0.4**	0.1ns
$\delta^{18}\text{O}_{\text{flag}}$ DM	0.1ns	-0.2ns	-0.4**	-0.3ns	0.1ns	-0.2ns	-0.3*	-0.3ns	-0.2ns	0.0ns	-0.5***	-0.1ns	-0.1ns	0.2ns	-0.2ns	0.2ns
$\delta^{18}\text{O}_{\text{flag}}$ WSF	-0.3*	0.0ns	-0.8***	-0.2ns	-0.2ns	-0.5**	-0.6***	-0.2ns	-0.7*	-0.3ns	-0.5***	-0.1ns	-0.6***	-0.2ns	-0.3*	0.1ns
$\delta^{18}\text{O}_{\text{ear}}$ DM	-0.1ns	0.2ns	-0.2ns	0.1ns	0.1ns	-0.3ns	-0.1ns	0.1ns	-0.1ns	-0.1ns	0.1ns	-0.2ns	-0.0ns	-0.0ns	-0.0ns	-0.1ns
$\delta^{18}\text{O}_{\text{ear}}$ WSF	-0.1ns	0.2ns	-0.3*	-0.3*	0.1ns	-0.4**	-0.1ns	-0.3*	-0.1ns	-0.2ns	-0.1ns	-0.1ns	-0.2ns	-0.1ns	-0.1ns	-0.1ns
$\delta^{18}\text{O}_{\text{grain}}$	0.1ns	0.1ns	-0.0ns	-0.0ns	-0.2ns	-0.2ns	-0.2ns	0.1ns	-0.2ns	-0.2ns	-0.1ns	0.1ns	-0.3*	-0.2ns	-0.1ns	-0.2ns
<b>Carbon</b>																
$\delta^{13}\text{C}_{\text{flag}}$ DM	-0.2ns	-0.7***	-0.7***	-0.59***	0.1ns	-0.3*	-0.6***	-0.6***	0.4s	0.3*	-0.6***	0.1ns	0.5***	0.4**	-0.1ns	0.1ns
$\delta^{13}\text{C}_{\text{flag}}$ WSF	-0.4**	-0.4**	-0.8***	-0.6***	-0.3ns	-0.3*	-0.6***	-0.6***	-0.5**	0.1ns	-0.6***	-0.1ns	-0.4**	0.2ns	-0.1ns	0.1ns
$\delta^{13}\text{C}_{\text{ear}}$ DM	-0.1ns	-0.5***	-0.7***	-0.5***	0.4**	-0.4**	-0.4**	-0.5***	0.5ns	0.4**	-0.3*	0.1ns	0.5***	0.4**	-0.1ns	0.2ns
$\delta^{13}\text{C}_{\text{ear}}$ WSF	-0.1ns	-0.3*	-0.6***	-0.42**	0.4**	-0.5***	-0.4**	-0.4**	0.4ns	0.3*	-0.2ns	0.1ns	0.2ns	0.3*	-0.1ns	0.2ns
$\delta^{13}\text{C}_{\text{grain}}$	-0.4**	-0.7***	-0.8***	-0.29*	-0.3*	-0.3*	-0.8***	-0.3*	0.21**	0.47***	-0.57***	0.3*	0.16ns	0.4**	-0.3*	0.3ns

1102

1103 **Table 5.** Stepwise analysis for the whole set of 9 genotypes per three replicates in 2010  
 1104 under supplemental irrigation (SI), rainfed conditions (RF), N fertilization (HN) and  
 1105 without N fertilization (LN), including all growing conditions (global) and a  
 1106 combination of support irrigation and N fertilization (SI+HN), support irrigation  
 1107 without N fertilization (SI-LN), rainfed conditions and N fertilization (RF+HN) and  
 1108 rainfed conditions without N fertilization (RF-LN) with *GY* as a dependent variable, and  
 1109 carbon ( $\delta^{13}\text{C}$ ) oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) isotope composition of mature kernels  
 1110 (grains), soluble organic matter of flag leaves (leaf WSF) and ears (ear WSF) and  
 1111 oxygen and hydrogen isotope composition of stem water (stemW) as independent  
 1112 variables. In the environmental effect the ‘global’ stepwise analysis represents values  
 1113 obtained from the average of the three replicates per genotype under each growing  
 1114 condition (108 plots, n=36); the ‘SI’ and ‘RF’ stepwise analyses represent values  
 1115 obtained from the average of three replicates per genotype including HN and LN  
 1116 conditions (n=18), the ‘HN’ and ‘LN’ stepwise analysis represent values obtained from  
 1117 the average of three replicates per genotype including SI and RF conditions (n=18).  
 1118 Level of significance:  $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.05$

	<b>Treatment</b>	<b>Variable Chosen</b>	<b><i>r</i></b>	<b><i>R</i><sup>2</sup></b>	<b>Significance</b>
<b>Environmental effect</b>	<b>Global</b>	$\delta^{13}\text{C}_{\text{grain}}$	0.71	0.51	0.000
		$\delta^{13}\text{C}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}$	0.83	0.70	0.000
		$\delta^{13}\text{C}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}, \delta^{13}\text{C}_{\text{flag WSF}}$	0.87	0.75	0.000
		$\delta^{13}\text{C}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}, \delta^{13}\text{C}_{\text{flag WSF}}, \delta^{13}\text{C}_{\text{ear WSF}}$	0.89	0.80	0.000
	<b>SI</b>	$\delta^2\text{H}_{\text{ear WSF}}$	0.75	0.57	0.000
		$\delta^2\text{H}_{\text{ear WSF}}, \delta^2\text{H}_{\text{stemW}}$	0.85	0.71	0.000
	<b>RF</b>	$\delta^2\text{H}_{\text{grain}}$	0.61	0.38	0.006
		$\delta^2\text{H}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}$	0.84	0.70	0.000
	<b>HN</b>	$\delta^{13}\text{C}_{\text{grain}}$	0.89	0.80	0.000
		$\delta^{13}\text{C}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}$	0.94	0.82	0.000
		$\delta^{13}\text{C}_{\text{grain}}, \delta^{18}\text{O}_{\text{flag WSF}}, \delta^{18}\text{O}_{\text{grain}}$	0.96	0.92	0.000
	<b>LN</b>	$\delta^{18}\text{O}_{\text{flag WSF}}$	0.76	0.58	0.000
		$\delta^{18}\text{O}_{\text{flag WSF}}, \delta^{13}\text{C}_{\text{grain}}$	0.84	0.70	0.000

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1123 **FIGURE LEGENDS**

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1125 **Fig. 1** Illustration of a wheat plant and the stable isotope composition (‰) of hydrogen  
1126 ( $\delta^2\text{H}$ ), oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) of different plant parts (flag leaves, ears and  
1127 roots) sampled at mid grain filling, plus mature kernels (grains) and the water (blue  
1128 drops) from the basal part of the stems, flag leaves and developing grains. Values  
1129 presented are means from the dry matter of five representative plants per plot and  
1130 including all treatments.  $\delta^{13}\text{C}$  DM was measured in 108 plots (five cultivars and four  
1131 landraces, four growing conditions and three replicates), whereas  $\delta^{18}\text{O}$  DM and  $\delta^2\text{H}$  DM  
1132 were measured in 48 plots (two cultivars and two landraces, four growing conditions  
1133 and three replicates) during the 2010 crop cycle. The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of the water  
1134 extracted from the flag leaves were analyzed in a subset of two cultivars and two  
1135 landraces (with three replicates) under fertilized conditions and two water regimes (18  
1136 plots) (landraces in SI conditions were discarded due to lodging).  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  were  
1137 measured in water from the stems, developing grains and dry matter in a subset of five  
1138 cultivars and five landraces (with three replicates) under fertilized conditions and two  
1139 water regimes (45 plots) (landraces were discarded due to lodging under SI conditions,  
1140 see Materials and Methods). Analyses of water extracted from different tissues were  
1141 performed in samples from the 2011 crop season.

1142

1143 **Fig. 2** Schematic representation of the major steps in the development of the ratios of  
1144 oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) isotope composition (‰) in plant carbohydrates and  
1145 tissue water from data obtained from the water-soluble fraction of the flag leaves and  
1146 ears (flag WSF, ears WSF) and water extracted from different plant tissues (grain water,  
1147 flag leaf water and stem water) at mid grain filling, plus mature kernels (grains) in nine  
1148 durum wheat genotypes and three replicates during the 2010 crop cycle. Each value  
1149 represents the mean  $\pm$  SD. Arrows represent the change in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  from water  
1150 sources (including irrigation water, soil water; precipitation water, Pp water) to  
1151 carbohydrates in autotrophic or heterotrophic tissues. White circles and arrows represent  
1152  $\delta^{18}\text{O}$ , black circles and arrows represent  $\delta^2\text{H}$ .

1153



1154 **Fig. 3** Linear regression of the relationship between the carbon ( $\delta^{13}\text{C}$ ) oxygen ( $\delta^{18}\text{O}$ )  
1155 and hydrogen ( $\delta^2\text{H}$ ) isotope compositions of the water-soluble fraction (WSF) within  
1156 the flag leaves (left column, closed circles), ears (middle column, open triangles) and  
1157 mature kernels (right column, open circles). Nine genotypes and three replicates per  
1158 genotype were considered, accounting for a total of 108 plot values under all growing  
1159 conditions of the 2010 crop season. Level of significance: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ;  
1160 \*,  $P < 0.05$ ; ns, not significant,  $P > 0.05$ .

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1162 **Fig. 4** Linear regression of the relationship of the carbon ( $\delta^{13}\text{C}$ ) oxygen ( $\delta^{18}\text{O}$ ) and  
1163 hydrogen ( $\delta^2\text{H}$ ) isotope compositions in mature kernels (grains) and in the dry matter  
1164 (DM) and water-soluble fraction (WSF) of the flag leaves and the ears with the grain  
1165 yield ( $GY$ ), the stomatal conductance ( $g_s$ ) and the total nitrogen concentration of the flag  
1166 leaves (N-Flag) and ears (N-Ear).  $\delta^{13}\text{C}$  DM and  $\delta^{13}\text{C}$  WSF,  $\delta^{18}\text{O}$  WSF and  $\delta^2\text{H}$  WSF  
1167 were measured in 108 plots (five cultivars and four landraces, four growing conditions  
1168 and three replicates per genotype and condition), whereas  $\delta^{18}\text{O}$  DM and  $\delta^2\text{H}$  DM were  
1169 measured in 48 plots (two cultivars and two landraces, four growing conditions and  
1170 three replicates) during the 2010 crop cycle. Analyses were performed in samples from  
1171 the 2010 crop season. Level of significance: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ;  
1172 ns, not significant,  $P > 0.05$ .

1173 **Fig. 5** Daily mean precipitation (mm), evapotranspiration (mm) and air temperature  
1174 ( $^{\circ}\text{C}$ ) during the growing season from flowering to physiological maturity expressed as  
1175 thermal time ( $^{\circ}\text{C}\cdot\text{day}$ ) during the 2010 (upper panel) and 2011 crop seasons (lower  
1176 panel). Vertical dotted lines symbolize sampling dates and vertical dashed lines  
1177 represent dates of irrigation.

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1185 **REFERENCES**

- 1186 Araus JL, Tapia L (1987) photosynthetic gas exchange characteristics of wheat flag leaf  
1187 blades and sheaths during grain filling: the case of a spring crop grown under  
1188 mediterranean climate conditions. *Plant Physiol* 85: 667–73
- 1189 Araus JL, Brown HR, Febrero A, Bort J, Serret MD (1993) Ear photosynthesis, carbon  
1190 isotope discrimination and the contribution of respiratory CO<sub>2</sub> to differences in  
1191 grain mass in durum wheat. *Plant, Cell Environ* 16: 383–392
- 1192 Araus JL, Amaro T, Casadesús J, Asbati A, Nachit MM (1998) Relationships between  
1193 ash content, carbon isotope discrimination and yield in durum wheat. *Aust J Plant*  
1194 *Physiol* 25: 835
- 1195 Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C3  
1196 cereals: what should we breed for? *Ann Bot* 89 Spec No: 925–40
- 1197 Araus JL, Villegas D, Aparicio N, del Moral LFG, El Hani S, Rharrabti Y, Ferrio JP,  
1198 Royo C (2003) Environmental factors determining carbon isotope discrimination  
1199 and yield in durum wheat under Mediterranean conditions. *Crop Sci* 43: 170–180
- 1200 Araus JL, Ferrio JP, Buxó R, Voltas J (2007) The historical perspective of dryland  
1201 agriculture: lessons learned from 10,000 years of wheat cultivation. *J Exp Bot* 58:  
1202 131–45
- 1203 Araus JL, Cabrera-Bosquet L, Serret MD, Bort J, Nieto-Taladriz MT (2013)  
1204 Comparative performance of  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  for phenotyping durum wheat  
1205 adaptation to a dryland environment. *Funct Plant Biol* 40: 595–608
- 1206 Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Funct*  
1207 *Plant Biol* 34: 83–94
- 1208 Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in  
1209 carbon and oxygen isotope ratios of cotton leaves. *Plant Cell Environ* 23: 473–485
- 1210 Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000) Oxygen isotope ratio of leaf  
1211 and grain material correlates with stomatal conductance and grain yield in irrigated  
1212 wheat. *Aust J Plant Physiol* 27: 625–637
- 1213 Barbour MM, Roden JS, Farquhar GD, Ehleringer JR (2004) Expressing leaf water and  
1214 cellulose oxygen isotope ratios as enrichment above source water reveals evidence

- 1215 of a Peclet effect. *Oecologia* 138: 426–435
- 1216 Barlow E, Lee J, Munns R, Smart M (1980) Water relations of the developing wheat  
1217 grain. *Aust J Plant Physiol.* doi: 10.1071/PP9800519
- 1218 Bloom AJ (2015) Photorespiration and nitrate assimilation: a major intersection  
1219 between plant carbon and nitrogen. *Photosynth Res* 123: 117–128
- 1220 Blum A (1985) Photosynthesis and transpiration in leaves and ears of wheat and barley  
1221 varieties. *J Exp Bot* 36: 432–440
- 1222 Bort J, Febrero A, Amaro T, Araus J (1994) Role of awns in ear water-use efficiency  
1223 and grain weight in barley. *Agronomie* 14: 133–139
- 1224 Bort J, Brown RH, Araus JL (1996) Refixation of respiratory CO<sub>2</sub> in the ears of C3  
1225 cereals. *J Exp Bot* 47: 1567–1575
- 1226 Bottinga Y, Craig H (1968) Oxygen isotope fractionation between CO<sub>2</sub> and water, and  
1227 the isotopic composition of marine atmospheric CO<sub>2</sub>. *Earth Planet Sci Lett* 5: 285–  
1228 295
- 1229 Buchanan BB, Gruissem W, Jones RL (2015) *Biochemistry & Molecular Biology of*  
1230 *Plants.* *J Chem Inf Model.* doi: 10.1017/CBO9781107415324.004
- 1231 Cabrera-Bosquet L, Molero G, Nogues S, Araus JL (2009a) Water and nitrogen  
1232 conditions affect the relationships of  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  to gas exchange and growth in  
1233 durum wheat. *J Exp Bot* 60: 1633–1644
- 1234 Cabrera-Bosquet L, Sanchez C, Araus JL (2009b) Oxygen isotope enrichment ( $\Delta^{18}\text{O}$ )  
1235 reflects yield potential and drought resistance in maize. *Plant Cell Environ* 32:  
1236 1487–1499
- 1237 Cabrera-Bosquet L, Albrizio R, Nogues S, Luis Araus J (2011) Dual  $\Delta^{13}\text{C} / \Delta^{18}\text{O}$   
1238 response to water and nitrogen availability and its relationship with yield in field-  
1239 grown durum wheat. *Plant Cell Environ* 34: 418–433
- 1240 Caley CY, Duffus CM, Jeffcoat B (1990) Photosynthesis in the pericarp of developing  
1241 wheat grains. *J Exp Bot* 41: 303–307
- 1242 Ceglar A, Toreti A, Lecerf R, Van der Velde M, Dentener F (2016) Impact of  
1243 meteorological drivers on regional inter-annual crop yield variability in France.  
1244 *Agric For Meteorol.* doi: 10.1016/j.agrformet.2015.10.004

- 1245 Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls  
1246 over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus*  
1247 *globulus*. *Tree Physiol* 25: 129–146
- 1248 Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD (2007) Transpiration  
1249 efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *J*  
1250 *Exp Bot* 58: 3549–66
- 1251 Cernusak LA, Mejia-Chang M, Winter K, Griffiths H (2008) Oxygen isotope  
1252 composition of CAM and C3 *Clusia* species: Non-steady-state dynamics control  
1253 leaf water  $^{18}\text{O}$  enrichment in succulent leaves. *Plant, Cell Environ.* doi:  
1254 10.1111/j.1365-3040.2008.01868.x
- 1255 Cernusak LA, Barbour MM, Arndt SK, Cheesman AW, English NB, Feild TS, Helliker  
1256 BR, Holloway-Phillips MM, Holtum JAM, Kahmen A, et al (2016) Stable isotopes  
1257 in leaf water of terrestrial plants. *Plant Cell Environ* 39: 1087–1102
- 1258 Chikaraishi Y, Naraoka H (2003) Compound-specific  $\delta\text{D}$ - $\delta^{13}\text{C}$  analyses of n-alkanes  
1259 extracted from terrestrial and aquatic plants. *Phytochemistry*. doi: 10.1016/S0031-  
1260 9422(02)00749-5
- 1261 Chikaraishi Y, Naraoka H, Poulson SR (2004) Carbon and hydrogen isotopic  
1262 fractionation during lipid biosynthesis in a higher plant (*Cryptomeria japonica*).  
1263 *Phytochemistry*. doi: 10.1016/j.phytochem.2003.12.003
- 1264 Cochrane MP, Duffus CM (1979) Morphology and ultrastructure of immature cereal  
1265 grains in relation to transport. *Ann Bot* 44: 67–72
- 1266 Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is  
1267 positively correlated with grain yield and dry matter production in field-grown  
1268 wheat<sup>1</sup>. *Crop Sci* 27: 996-1001
- 1269 Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-  
1270 use efficiency. *J Exp Bot* 55: 2447–2460
- 1271 Coplen TB (1988) Normalization of oxygen and hydrogen isotope data. *Chem Geol Isot*  
1272 *Geosci Sect* 72: 293–297
- 1273 Cormier M-A, Werner RA, Sauer PE, Gröcke DR, Leuenberger MC, Wieloch T,  
1274 Schleucher J, Kahmen A (2018)  $^2\text{H}$ -fractionations during the biosynthesis of  
1275 carbohydrates and lipids imprint a metabolic signal on the  $\delta^2\text{H}$  values of plant

- 1276 organic compounds. *New Phytol* 218: 479–491
- 1277 Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and the  
1278 marine atmosphere. In E Tongiorgi, ed, *Proc. a Conf. Stable Isot. Oceanogr. Stud.*  
1279 *Palaeotemperatures*. Lischi and Figli, Pisa, pp 9–130
- 1280 Cuntz M, Ogée J, Farquhar GD, Peylin P, Cernusak LA (2007) Modelling advection  
1281 and diffusion of water isotopologues in leaves. *Plant Cell Environ* 30: 892–909
- 1282 Dansgaard W (1964) Stable isotopes in precipitation. *Tellus* 16: 436–468
- 1283 Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the “woody” tissues  
1284 of plants: Implications for plant water source, water uptake, and other studies  
1285 which use the stable isotopic composition of cellulose. *Geochim Cosmochim Acta*  
1286 57: 3487–3492
- 1287 Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in  
1288 Plant Ecology. *Annu Rev Ecol Syst* 33: 507–559
- 1289 Epstein S, Yapp CJ, Hall JH (1976) The determination of the D/H ratio of non-  
1290 exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth*  
1291 *Planet Sci Lett* 30: 241–251
- 1292 Epstein S, Thompson P, Yapp CJ (1977) Oxygen and hydrogen isotopic ratios in plant  
1293 cellulose. *Science* 198: 1209–15
- 1294 Farquhar G (1989) Carbon isotope discrimination and photosynthesis. *Annu Rev Plant*  
1295 *Physiol Plant Mol Biol* 40: 503–537
- 1296 Farquhar GD, Oleary MH, Berry JA (1982) on the relationship between carbon isotope  
1297 discrimination and the inter-cellular carbon-dioxide concentration in leaves. *Aust J*  
1298 *Plant Physiol* 9: 121–137
- 1299 Farquhar G, Richards R (1984) Isotopic composition of plant carbon correlates with  
1300 water-use efficiency of wheat genotypes. *Aust J Plant Physiol* 11: 539–552
- 1301 Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of  
1302 carbon dioxide between terrestrial plants and the atmosphere. In A Ehleringer, J  
1303 Hal, G Farquhar, eds, *Stable Isot. Plant CarbonWater Relations*, Academic P. San  
1304 Diego, pp 47–70
- 1305 Farquhar G, Barbour MM, Henry BK (1998) Interpretation of oxygen isotope

- 1306 composition of leaf material. In HG Griffiths, ed, *Stable Isot.*, Bios Scien. Oxford,  
1307 pp 27–62
- 1308 Farquhar GD, Gan KS (2003) On the progressive enrichment of the oxygen isotopic  
1309 composition of water along a leaf (Reprinted from *Plant, Cell and Environment*,  
1310 vol 26, pg 801-819, 2003). *Plant Cell Environ* 26: 1579–1597
- 1311 Farquhar GD, Cernusak LA, Barnes B (2007) Heavy water fractionation during  
1312 transpiration. *Plant Physiol* 143: 11–18
- 1313 Feakins SJ, Sessions AL (2010) Controls on the D/H ratios of plant leaf waxes in an  
1314 arid ecosystem. *Geochim Cosmochim Acta* 74: 2128–2141
- 1315 Ferrio JP, Mateo MA, Bort J, et al (2007) Relationships of grain  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  with  
1316 wheat phenology and yield under water-limited conditions. *Ann Appl Biol*  
1317 150:207-215. doi: 10.1111/j.1744-7348.2007.00115.x
- 1318 Fischer RA, Rees D, Sayre KD, Lu Z-M, Condon AG, Saavedra AL (1998) Wheat  
1319 yield progress associated with higher stomatal conductance and photosynthetic  
1320 rate, and cooler canopies. *Crop Sci* 38: 1467–1475
- 1321 Filot MS, Leuenberger M, Pazdur A, Boettger T (2006) Rapid online equilibration  
1322 method to determine the D/H ratios of non-exchangeable hydrogen in cellulose.  
1323 *Rapid Commun Mass Spectrom* 20: 3337–3344
- 1324 Foyer CH, Neukermans J, Queval G, Noctor G, Harbinson J (2012) Photosynthetic  
1325 control of electron transport and the regulation of gene expression. *J Exp Bot* 63:  
1326 1637–1661
- 1327 Food and Agriculture Organization of the United Nations. 2017. Available at  
1328 <http://www.fao.org/home/en/> (accessed 25 July 2017).
- 1329 Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, Monson RK (2014) Stable  
1330 isotopes in tree rings: towards a mechanistic understanding of isotope fractionation  
1331 and mixing processes from the leaves to the wood. *Tree Physiol* 34: 1–23
- 1332 Gonfiantini R (1978) Standards for stable isotope measurements in natural compounds.  
1333 *Nature* 271: 534–536
- 1334 Gonfiantini R, Gratziu S, Tongiorgi E (1965) Oxygen isotopic composition of water in  
1335 leaves. *Isot Radiat Soil Plant Nutr Stud. IAEA, Vienna*, pp 405–410
- 1336 Gonfiantini MM (2007) Stable oxygen isotope composition of plant tissue: a review.

- 1337       Funct Plant Biol 34: 83–94
- 1338   Hayes JM (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic  
1339       processes. *Rev Mineral Geochemistry* 43: 225–277
- 1340   Helliker BR, Ehleringer JR (2000) Establishing a grassland signature in veins: O-18 in  
1341       the leaf water of C<sub>3</sub> and C<sub>4</sub> grasses. *Proc Natl Acad Sci U S A* 97: 7894–7898
- 1342   Helliker BR, Ehleringer JR (2002) Differential <sup>18</sup>O enrichment of leaf cellulose in C<sub>3</sub>  
1343       versus C<sub>4</sub> grasses. *Funct Plant Biol* 29: 435–442
- 1344   Hoganson CW, Babcock GT (1997) A metalloradical mechanism for the generation of  
1345       oxygen from water in photosynthesis. *Science* 277: 1953–1956
- 1346   Hou J, D’Andrea WJ, MacDonald D, Huang Y (2007) Hydrogen isotopic variability in  
1347       leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts  
1348       (USA). *Org Geochem* 38: 977–984
- 1349   Hubick KT, Hammer GL, Farquhar GD, Wade LJ, von Caemmerer S, Henderson SA  
1350       (1990) Carbon isotope discrimination varies genetically in C<sub>4</sub> species. *Plant*  
1351       *Physiol* 92: 534–537
- 1352   Jensen A, Lorenzen B, Østergaard HS, Hvelplund EK (1990) Radiometric estimation of  
1353       biomass and nitrogen content of barley grown at different nitrogen levels†. *Int J*  
1354       *Remote Sens* 11: 1809–1820
- 1355   Jia S, Lv J, Jiang S, Liang T, Liu C, Jing Z (2015) Response of wheat ear  
1356       photosynthesis and photosynthate carbon distribution to water deficit.  
1357       *Photosynthetica* 53: 95–109
- 1358   Kahmen A, Schefuß E, Sachse D (2013) Leaf water deuterium enrichment shapes leaf  
1359       wax n-alkane δD values of angiosperm plants I: Experimental evidence and  
1360       mechanistic insights. *Geochim Cosmochim Acta* 111: 39–49
- 1361   Knoppik D, Selinger H, Ziegler-Jons A (1986) Differences between the flag leaf and the  
1362       ear of a spring wheat cultivar (*Triticum aestivum* cv. Arkas) with respect to the  
1363       CO<sub>2</sub> response of assimilation, respiration and stomatal conductance. *Physiol Plant*  
1364       68: 451–457
- 1365   Li X, Wang H, Li H, Zhang L, Teng N, Lin Q, Wang J, Kuang T, Li Z, Li B, et al  
1366       (2006) Awns play a dominant role in carbohydrate production during the grain-  
1367       filling stages in wheat (*Triticum aestivum*). *Physiol Plant* 127: 701–709

- 1368 Liu H, Guo B, Wei Y, Wei S, Ma Y, Zhang W (2015) Effects of region, genotype,  
1369 harvest year and their interactions on  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta\text{D}$  in wheat kernels. *Food*  
1370 *Chem* 171: 56–61
- 1371 Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008)  
1372 Prioritizing climate change adaptation needs for food security in 2030. *Science*  
1373 319: 607–610
- 1374 Lopes MS, Reynolds MP (2010) Dissecting drought adaptation into its phenotypic and  
1375 genetic components in wheat. *Asp Appl Biol* 7–11
- 1376 Luo Y, Sternberg L (1991) Deuterium heterogeneity in starch and cellulose nitrate of  
1377 cam and  $\text{C}_3$  plants. *Phytochemistry* 30: 1095–1098
- 1378 Luo Y-H, Steinberg L, Suda S, Kumazawa S, Mitsui A (1991) Extremely Low D/H  
1379 ratios of photoproducted hydrogen by cyanobacteria. *Plant Cell Physiol* 32: 897–  
1380 900
- 1381 Martín-Gómez P, Barbeta A, Voltas J, Peñuelas J, Dennis K, Palacio S, Dawson TE,  
1382 Ferrio JP (2015) Isotope-ratio infrared spectroscopy: a reliable tool for the  
1383 investigation of plant-water sources? *New Phytol* 207: 914–927
- 1384 Monneveux P, Reynolds MP, Trethowan R, González-Santoyo H, Peña RJ, Zapata F  
1385 (2005) Relationship between grain yield and carbon isotope discrimination in  
1386 bread wheat under four water regimes. *Eur J Agron* 22: 231–242
- 1387 Moore FC, Lobell DB (2015) The fingerprint of climate trends on European crop yields.  
1388 *Proc Natl Acad Sci*. doi: 10.1073/pnas.1409606112
- 1389 Offermann C, Pedro Ferrio J, Holst J, Grote R, Siegwolf R, Kayler Z, Gessler A (2011)  
1390 The long way down-are carbon and oxygen isotope signals in the tree ring  
1391 uncoupled from canopy physiological processes? *Tree Physiol* 31: 1088–1102
- 1392 Oweis T, Pala M, Ryan J (1998) Stabilizing rainfed wheat yields with supplemental  
1393 irrigation and nitrogen in a mediterranean climate. *Agron J* 90: 672
- 1394 Pande P, Datta P, Bhattacharya S (1994) Biphasic enrichment of  $\text{H}_2^{18}\text{O}$  in developing  
1395 wheat grain water. *Indian J Exp Biol* 37: 30–31
- 1396 Pande P, Datta P, Bhattacharya S, Tyagi S (1995) Post-anthesis metabolic-enrichment  
1397 of  $\text{H}_2^{18}\text{O}$  in wheat grain. *Indian J Exp Biol* 33: 394–396



- 1398 Qi H, Coplen TB (2011) Investigation of preparation techniques for  $\delta^2\text{H}$  analysis of  
1399 keratin materials and a proposed analytical protocol. *Rapid Commun Mass*  
1400 *Spectrom* 25: 2209–2222
- 1401 Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant*  
1402 *Growth Regul* 20: 157–166
- 1403 Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding  
1404 opportunities for increasing the efficiency of water use and crop yield in temperate  
1405 cereals. *Crop Sci* 42: 111–121
- 1406 Rieder S V., Rose IA (1959) The mechanism of the triosephosphate isomerase reaction.  
1407 *J. Biol. Chem.* 234: 1007-1010
- 1408 Roden JS, Lin G, Ehleringer JR (2000) A mechanistic model for interpretation of  
1409 hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim Cosmochim*  
1410 *Acta* 64: 21–35
- 1411 Sachse D, Radke J, Gleixner G (2006)  $\delta\text{D}$  values of individual n-alkanes from terrestrial  
1412 plants along a climatic gradient - Implications for the sedimentary biomarker  
1413 record. *Org Geochem.* doi: 10.1016/j.orggeochem.2005.12.003
- 1414 Sachse D, Billault I, Bowen GJ, Chikaraishi Y, Dawson TE, Feakins SJ, Freeman KH,  
1415 Magill CR, McInerney FA, van der Meer MTJ, et al (2012) Molecular  
1416 paleohydrology: Interpreting the hydrogen-isotopic composition of lipid  
1417 biomarkers from photosynthesizing organisms. *Annu Rev Earth Planet Sci* 40:  
1418 221–249
- 1419 Sadras VO (2004) Yield and water-use efficiency of water- and nitrogen-stressed wheat  
1420 crops increase with degree of co-limitation. *Eur J Agron* 21: 455–464
- 1421 Salazar-Tortosa D, Castro J, Villar-Salvador P, et al (2018) The "isohydric trap": a  
1422 proposed feedback between water shortage, stomatal regulation and nutrient  
1423 acquisition drives differential growth and survival of European pines under  
1424 climatic dryness. *Glob Chang Biol.* doi: 10.1111/gcb.14311
- 1425 Sanchez-Bragado R, Elazab A, Zhou B, Serret MD, Bort J, Nieto-Taladriz MT, Araus  
1426 JL (2014a) Contribution of the ear and the flag leaf to grain filling in durum wheat  
1427 inferred from the carbon isotope signature: genotypic and growing conditions  
1428 effects. *J Integr Plant Biol* 56: 444–454

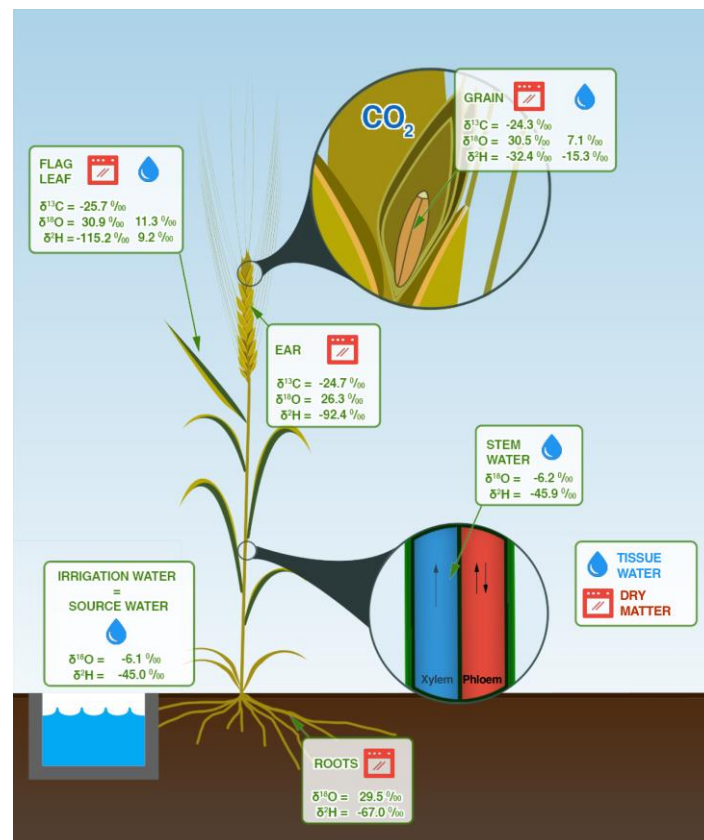
- 1429 Sánchez-Bragado R, Araus JL, Scheerer U, Cairns JE, Rennenberg H, Ferrio JP (2016)  
1430 Factors preventing the performance of oxygen isotope ratios as indicators of grain  
1431 yield in maize. *Planta*. doi: 10.1007/s00425-015-2411-4
- 1432 Sauer PE, Schimmelmann A, Sessions AL, Topalov K (2009) Simplified batch  
1433 equilibration for D/H determination of non-exchangeable hydrogen in solid organic  
1434 material. *Rapid Commun Mass Spectrom* 23: 949–956
- 1435 Schimmelmann A (1991) Determination of the concentration and stable isotopic  
1436 composition of nonexchangeable hydrogen in organic matter. *Anal Chem*. doi:  
1437 10.1021/ac00021a013
- 1438 Schimmelmann A, Lawrence M, Michael E (2001) Stable isotopes ratios of organic H,  
1439 C and N in the Miocene Monterey Formation, California. In CM Isaac, J  
1440 Rullkötter, eds, *Monterey Form. From rocks to Mol.* Columbia University Press,  
1441 pp 86–106
- 1442 Schleucher J, Vanderveer P, Markley JL, Sharkey TD (1999) Intramolecular deuterium  
1443 distributions reveal disequilibrium of chloroplast phosphoglucose isomerase. *Plant,*  
1444 *Cell Environ*. doi: 10.1046/j.1365-3040.1999.00440.x
- 1445 Schmidt HL, Werner RA, Eisenreich W (2003) Systematics of <sup>2</sup>H patterns in natural  
1446 compounds and its importance for the elucidation of biosynthetic pathways.  
1447 *Phytochem Rev* 2: 61–85
- 1448 Schmidt HL, Robins RJ, Werner RA (2015) Multi-factorial in vivo stable isotope  
1449 fractionation: causes, correlations, consequences and applications. *Isotopes*  
1450 *Environ Health Stud*. doi: 10.1080/10256016.2015.1014355
- 1451 Schwendenmann L, Pendall E, Sanchez-Bragado R, Kunert N, Hölscher D (2015) Tree  
1452 water uptake in a tropical plantation varying in tree diversity: interspecific  
1453 differences, seasonal shifts and complementarity. *Ecohydrology* 8: 1–12
- 1454 Serret M., Ortiz-Monasterio I, Pardo A, Araus J. (2008) The effects of urea fertilisation  
1455 and genotype on yield, nitrogen use efficiency,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in wheat. *Ann Appl*  
1456 *Biol* 153: 243–257
- 1457 Shangguan ZP, Shao MA, Dyckmans J (2000) Nitrogen nutrition and water stress  
1458 effects on leaf photosynthetic gas exchange and water use efficiency in winter  
1459 wheat. *Environ Exp Bot* 44: 141–149

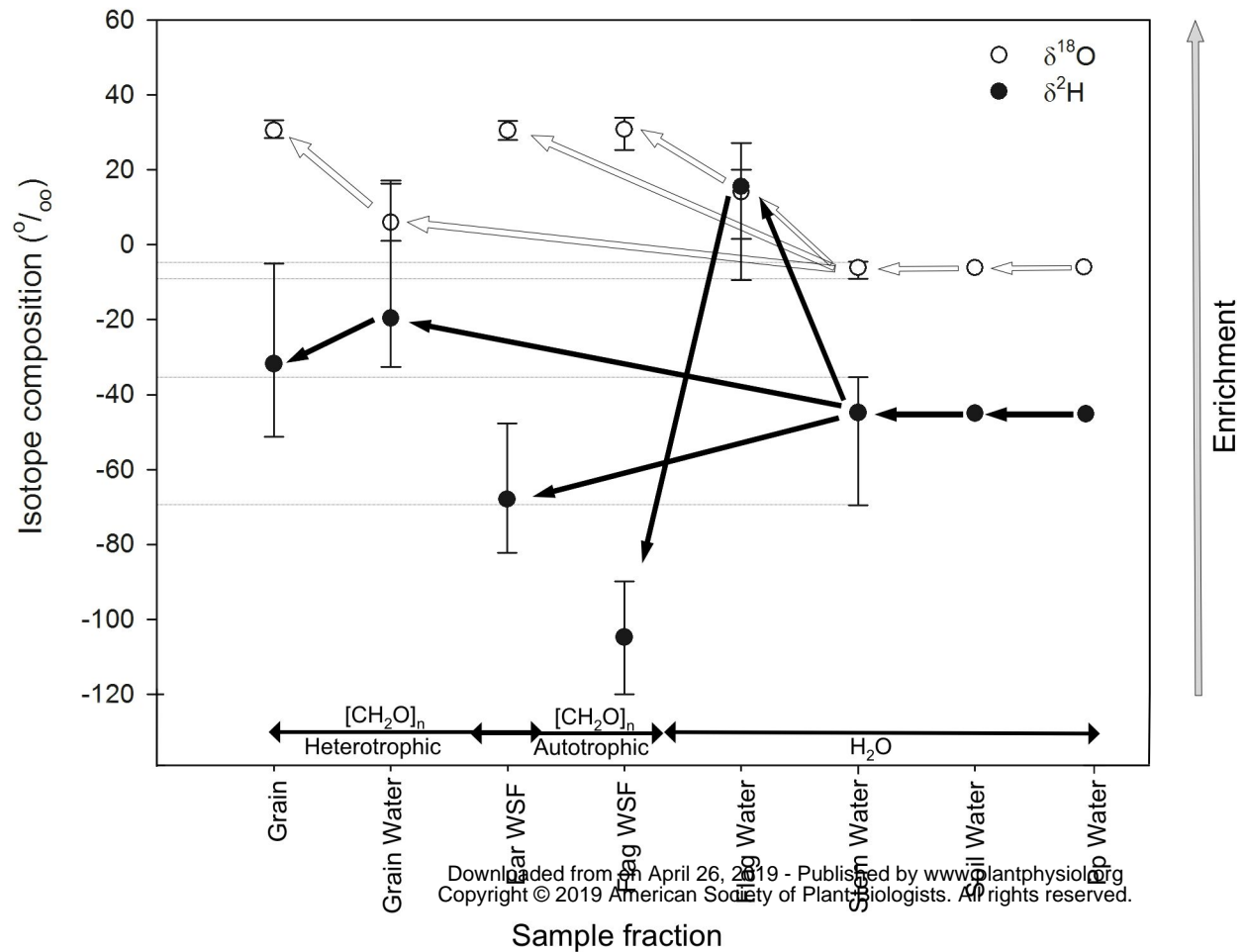
- 1460 Sessions AL, Burgoyne TW, Schimmelmann A, Hayes JM (1999) Fractionation of  
1461 hydrogen isotopes in lipid biosynthesis. *Org Geochem*. doi: 10.1016/S0146-  
1462 6380(99)00094-7
- 1463 Smith FA, Freeman KH (2006) Influence of physiology and climate on  $\delta D$  of leaf wax  
1464 n-alkanes from  $C_3$  and  $C_4$  grasses. *Geochim Cosmochim Acta*. doi:  
1465 10.1016/j.gca.2005.11.006
- 1466 Song X, Farquhar GD, Gessler A, Barbour MM (2014) Turnover time of the non-  
1467 structural carbohydrate pool influences  $\delta^{18}O$  of leaf cellulose. *Plant Cell Environ*.  
1468 doi: 10.1111/pce.12309
- 1469 Sternberg L, Deniro MJ (1983) Isotopic Composition of Cellulose from  $C_3$ ,  $C_4$ , and  
1470 CAM plants growing near one another. *Science* 220: 947–9
- 1471 Sternberg LO, Deniro MJ, Johnson HB (1984) Isotope ratios of cellulose from plants  
1472 having different photosynthetic pathways. *Plant Physiol* 74: 557–561
- 1473 Sternberg L, Deniro M, Savidge R (1986) Oxygen isotope exchange between  
1474 metabolites and water during biochemical reactions leading to cellulose synthesis.  
1475 *Plant Physiol* 82: 423–427
- 1476 Sternberg L (1988) D/H ratios of environmental water recorded by D/H ratios of plant  
1477 lipids. *Nature* 333: 59–61
- 1478 Tambussi EA, Nogués S, Araus JL (2005) Ear of durum wheat under water stress: water  
1479 relations and photosynthetic metabolism. *Planta* 221: 446–58
- 1480 Voltas J, Romagosa I, Lafarga A, Armesto AP, Araus JL, Sombrero A (1999) Genotype  
1481 by environment interaction for grain yield and carbon isotope discrimination of  
1482 barley in Mediterranean Spain. *Aust J Agric Res* 50: 1263–1271
- 1483 Wassenaar LI, Hobson KA (2000) Improved method for determining the stable-  
1484 hydrogen isotopic composition ( $\delta D$ ) of complex organic materials of  
1485 environmental interest. doi: 10.1021/ES990804I
- 1486 Winter K, Garcia M, Holtum JAM (2008) On the nature of facultative and constitutive  
1487 CAM: environmental and developmental control of CAM expression during early  
1488 growth of *Clusia*, *Kalanchoë*, and *Opuntia*. *J Exp Bot* 59: 1829–40
- 1489 Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants:

- 1490 powerful tools for unravelling the functional elements of CAM photosynthesis. *J*  
1491 *Exp Bot* 65: 3425–41
- 1492 Williams DG, Coltrain JB, Lott M, English NB, Ehleringer JR (2005) Oxygen isotopes  
1493 in cellulose identify source water for archaeological maize in the American  
1494 Southwest. *J Archaeol Sci* 32: 931–939
- 1495 Yakir D (1992) Variations in the natural abundance of oxygen-18 and deuterium in  
1496 plant carbohydrates. *Plant, Cell Environ* 15: 1005–1020
- 1497 Yakir D, Deniro MJ (1990) Oxygen and hydrogen isotope fractionation during cellulose  
1498 metabolism in *Lemna gibba* L. *Plant Physiol* 93: 325–332
- 1499 Yakir D, Deniro MJ, Ephrath JE (1990a) Effects of water-stress on oxygen, hydrogen  
1500 and carbon isotope ratios in 2 species of cotton plants. *Plant Cell Environ* 13: 949–  
1501 955
- 1502 Yakir D, Deniro MJ, Gat JR (1990b) Natural deuterium and oxygen-18 enrichment in  
1503 leaf water of cotton plants grown under wet and dry conditions - evidence for water  
1504 compartmentation and its dynamics. *Plant Cell Environ* 13: 49–56
- 1505 Yang H, Leng Q (2009) Molecular hydrogen isotope analysis of living and fossil  
1506 plants—*Metasequoia* as an example. *Prog Nat Sci* 19: 901–912
- 1507 Yousfi S, Serret MD, Araus JL (2013) Comparative response of  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  in  
1508 durum wheat exposed to salinity at the vegetative and reproductive stages. *Plant*  
1509 *Cell Environ* 36: 1214–27
- 1510 Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of  
1511 cereals. *Weed Res* 14: 415–421
- 1512 Zhao LJ, Xiao HL, Liu XH (2007) Relationships between carbon isotope discrimination  
1513 and yield of spring wheat under different water and nitrogen levels. *J Plant Nutr*  
1514 30: 947–963
- 1515 Zhou Y, Stuart-Williams H, Farquhar GD, Hocart CH (2010) The use of natural  
1516 abundance stable isotopic ratios to indicate the presence of oxygen-containing  
1517 chemical linkages between cellulose and lignin in plant cell walls. *Phytochemistry*.  
1518 doi: 10.1016/j.phytochem.2010.03.001
- 1519 Zhou Y, Grice K, Chikaraishi Y, Stuart-Williams H, Farquhar GD, Ohkouchi N (2011)  
1520 Temperature effect on leaf water deuterium enrichment and isotopic fractionation

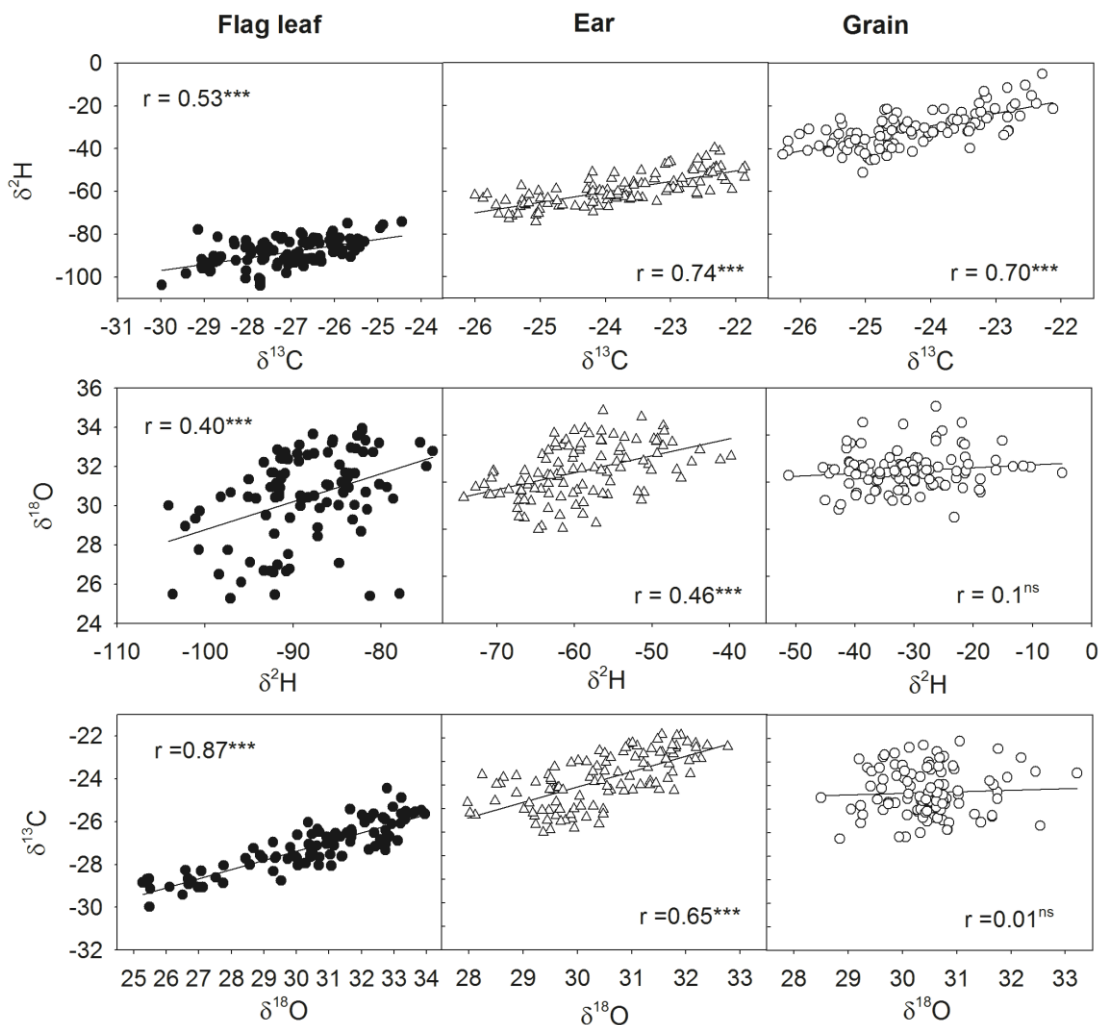
- 1521 during leaf lipid biosynthesis: Results from controlled growth of C<sub>3</sub> and C<sub>4</sub> land  
1522 plants. *Phytochemistry*. doi: 10.1016/j.phytochem.2010.10.022
- 1523 Zhou Y, Zhang B, Stuart-Williams H, Grice K, Hocart CH, Gessler A, Kayler ZE,  
1524 Farquhar GD (2018) On the contributions of photorespiration and  
1525 compartmentation to the contrasting intramolecular <sup>2</sup>H profiles of C<sub>3</sub> and C<sub>4</sub> plant  
1526 sugars. *Phytochemistry*. doi: 10.1016/j.phytochem.2017.11.004
- 1527 Ziegler H (1989) Stable Isotopes in Ecological research, Ecological. In: Rundel P,  
1528 Ehleringer JR, Nagy K, eds. Berlin: Springer-Verlag.
- 1529 Ziegler H, Osmond CB, Stichler W, Trimborn P (1976) Hydrogen isotope  
1530 discrimination in higher plants: Correlations with photosynthetic pathway and  
1531 environment. *Planta* 128: 85–92
- 1532
- 1533
- 1534

**Fig. 1** Illustration of a wheat plant and the stable isotope composition (‰) of hydrogen ( $\delta^2\text{H}$ ), oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) of different plant parts (flag leaves, ears and roots) sampled at mid grain filling, plus mature kernels (grains) and the water (blue drops) from the basal part of the stems, flag leaves and developing grains. Values presented are means from the dry matter of five representative plants per plot and including all treatments.  $\delta^{13}\text{C}$  DM was measured in 108 plots (five cultivars and four landraces, four growing conditions and three replicates), whereas  $\delta^{18}\text{O}$  DM and  $\delta^2\text{H}$  DM were measured in 48 plots (two cultivars and two landraces, four growing conditions and three replicates) during the 2010 crop cycle. The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of the water extracted from the flag leaves were analyzed in a subset of 2 cultivars and 2 landraces (with three replicates) under fertilized conditions and two water regimes (18 plots) (landraces in SI conditions were discarded due to lodging).  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  were measured in water from the stems, developing grains and dry matter in a subset of 5 cultivars and 5 landraces (with three replicates) under fertilized conditions and two water regimes (45 plots) (landraces were discarded due to lodging under SI conditions, see Materials and Methods). Analyses of water extracted from different tissues were performed in samples from the 2011 crop season.

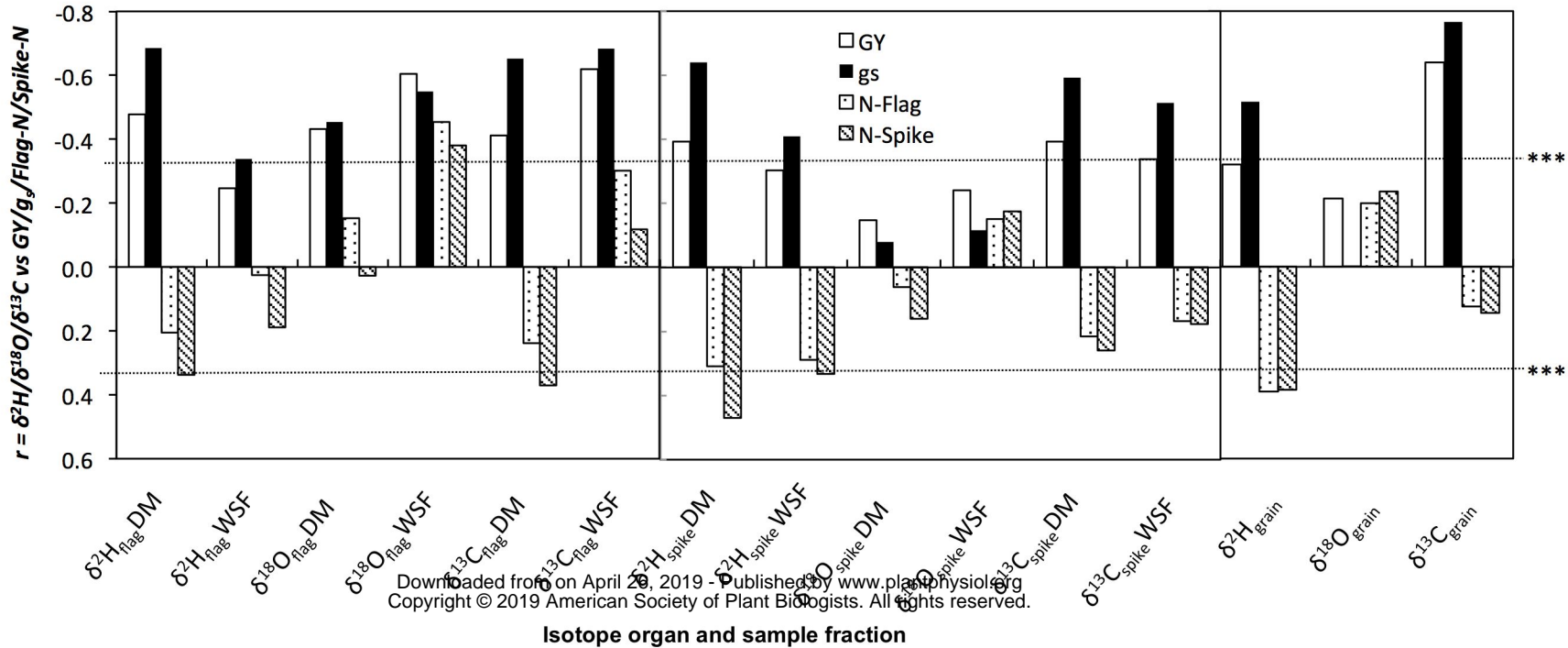




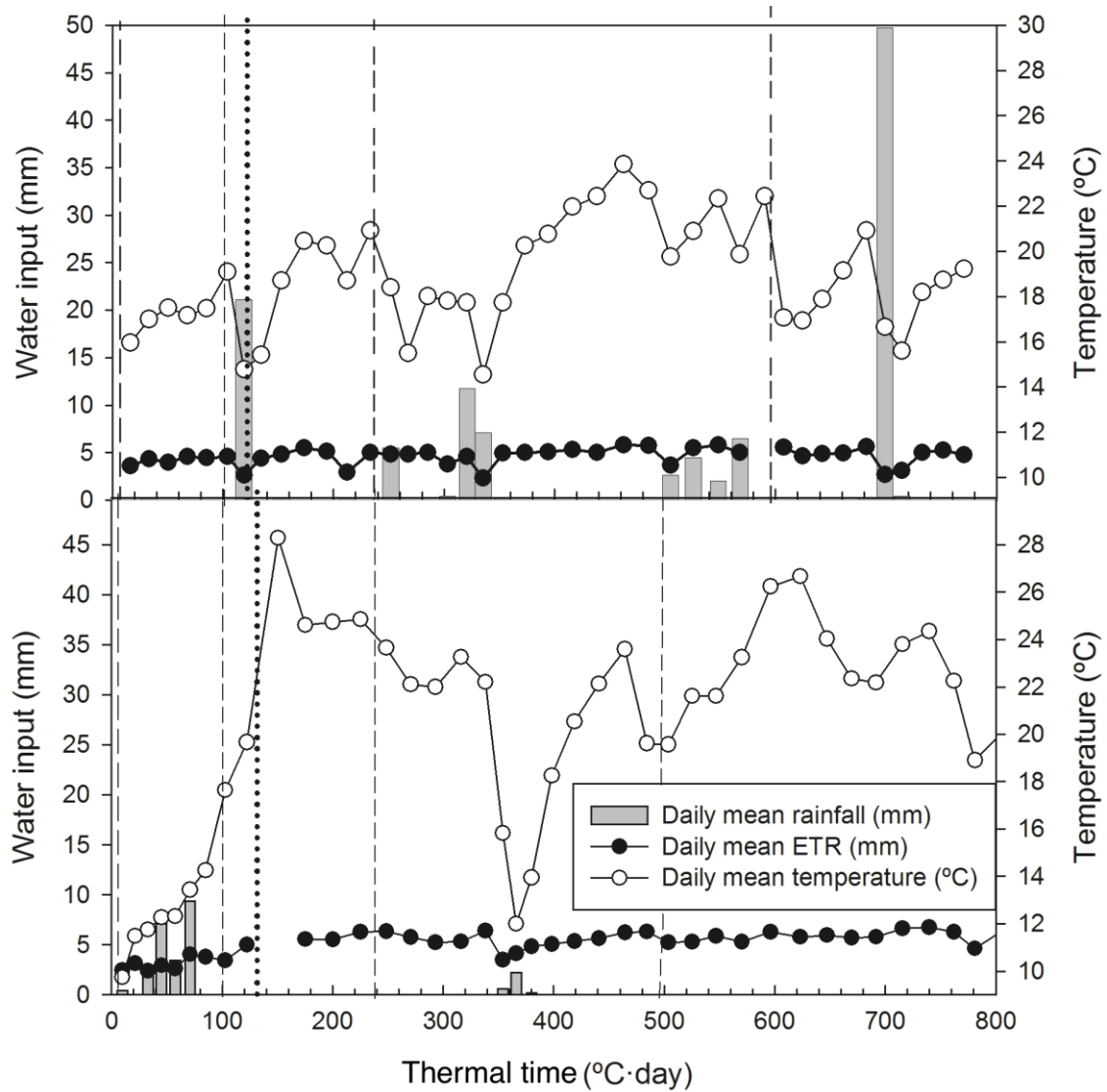
**Fig. 3** Linear regression of the relationship between the carbon ( $\delta^{13}\text{C}$ ) oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) isotope compositions of the water-soluble fraction (WSF) within the flag leaves (left column, closed circles), ears (middle column, open triangles) and mature kernels (right column, open circles). Nine genotypes and three replicates per genotype were considered, accounting for a total of 108 plot values under all growing conditions of the 2010 crop season. Level of significance: \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; ns, not significant,  $P > 0.05$ .







**Fig. 5** Daily mean precipitation (mm), evapotranspiration (mm) and air temperature ( $^{\circ}\text{C}$ ) during the growing season from flowering to physiological maturity expressed as thermal time ( $^{\circ}\text{C}\cdot\text{day}$ ) during the 2010 (upper panel) and 2011 crop seasons (lower panel). Vertical dotted lines symbolize sampling dates and vertical dashed lines represent dates of irrigation.



## Parsed Citations

**Araus JL, Tapia L (1987) photosynthetic gas exchange characteristics of wheat flag leaf blades and sheaths during grain filling: the case of a spring crop grown under mediterranean climate conditions. *Plant Physiol* 85: 667–73**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Araus JL, Brown HR, Febrero A, Bort J, Serret MD (1993) Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO<sub>2</sub> to differences in grain mass in durum wheat. *Plant, Cell Environ* 16: 383–392**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Araus JL, Amaro T, Casadesús J, Asbati A, Nachit MM (1998) Relationships between ash content, carbon isotope discrimination and yield in durum wheat. *Aust J Plant Physiol* 25: 835**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C<sub>3</sub> cereals: what should we breed for? *Ann Bot* 89 Spec No: 925–40**

**Araus JL, Villegas D, Aparicio N, del Moral LFG, El Hani S, Rharrabti Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. *Crop Sci* 43: 170–180**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Araus JL, Ferrio JP, Buxó R, Voltas J (2007) The historical perspective of dryland agriculture: lessons learned from 10,000 years of wheat cultivation. *J Exp Bot* 58: 131–45**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Araus JL, Cabrera-Bosquet L, Serret MD, Bort J, Nieto-Taladriz MT (2013) Comparative performance of  $\delta^{13}C$ ,  $\delta^{18}O$  and  $\delta^{15}N$  for phenotyping durum wheat adaptation to a dryland environment. *Funct Plant Biol* 40: 595–608**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Funct Plant Biol* 34: 83–94**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant Cell Environ* 23: 473–485**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Aust J Plant Physiol* 27: 625–637**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Barbour MM, Roden JS, Farquhar GD, Ehleringer JR (2004) Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. *Oecologia* 138: 426–435**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Barlow E, Lee J, Munns R, Smart M (1980) Water relations of the developing wheat grain. *Aust J Plant Physiol*. doi: 10.1071/PP9800519**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Bloom AJ (2015) Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. *Photosynth Res* 123: 117–128**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Blum A (1985) Photosynthesis and transpiration in leaves and ears of wheat and barley varieties. *J Exp Bot* 36: 432–440**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Bort J, Febrero A, Amaro T, Araus J (1994) Role of awns in ear water-use efficiency and grain weight in barley. *Agronomie* 14: 133–139**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Bort J, Brown RH, Araus JL (1996) Refixation of respiratory CO<sub>2</sub> in the ears of C<sub>3</sub> cereals. *J Exp Bot* 47: 1567–1575**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

- Bottinga Y, Craig H (1968) Oxygen isotope fractionation between CO<sub>2</sub> and water, and the isotopic composition of marine atmospheric CO<sub>2</sub>. Earth Planet Sci Lett 5: 285–295**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Buchanan BB, Gruissem W, Jones RL (2015) Biochemistry & Molecular Biology of Plants. J Chem Inf Model. doi: 10.1017/CBO9781107415324.004**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cabrera-Bosquet L, Molero G, Nogues S, Araus JL (2009a) Water and nitrogen conditions affect the relationships of  $\Delta^{13}\text{C}$  and  $\Delta^{18}\text{O}$  to gas exchange and growth in durum wheat. J Exp Bot 60: 1633–1644**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cabrera-Bosquet L, Sanchez C, Araus JL (2009b) Oxygen isotope enrichment ( $\Delta^{18}\text{O}$ ) reflects yield potential and drought resistance in maize. Plant Cell Environ 32: 1487–1499**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cabrera-Bosquet L, Albrizio R, Nogues S, Luis Araus J (2011) Dual  $\Delta^{13}\text{C}$  /  $\Delta^{18}\text{O}$  response to water and nitrogen availability and its relationship with yield in field-grown durum wheat. Plant Cell Environ 34: 418–433**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Caley CY, Duffus CM, Jeffcoat B (1990) Photosynthesis in the pericarp of developing wheat grains. J Exp Bot 41: 303–307**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ceglar A, Toreti A, Lecerf R, Van der Velde M, Dentener F (2016) Impact of meteorological drivers on regional inter-annual crop yield variability in France. Agric For Meteorol. doi: 10.1016/j.agrformet.2015.10.004**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, Eucalyptus globulus. Tree Physiol 25: 129–146**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cernusak LA, Winter K, Aranda J, Turner BL, Marshall JD (2007) Transpiration efficiency of a tropical pioneer tree (Ficus insipida) in relation to soil fertility. J Exp Bot 58: 3549–66**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cernusak LA, Mejia-Chang M, Winter K, Griffiths H (2008) Oxygen isotope composition of CAM and C<sub>3</sub>Clusia species: Non-steady-state dynamics control leaf water  $\delta^{18}\text{O}$  enrichment in succulent leaves. Plant, Cell Environ. doi: 10.1111/j.1365-3040.2008.01868.x**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cernusak LA, Barbour MM, Arndt SK, Cheesman AW, English NB, Feild TS, Helliker BR, Holloway-Phillips MM, Holtum JAM, Kahmen A, et al (2016) Stable isotopes in leaf water of terrestrial plants. Plant Cell Environ 39: 1087–1102**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chikaraishi Y, Naraoka H (2003) Compound-specific  $\delta\text{D}$ - $\delta^{13}\text{C}$  analyses of n-alkanes extracted from terrestrial and aquatic plants. Phytochemistry. doi: 10.1016/S0031-9422(02)00749-5**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chikaraishi Y, Naraoka H, Poulson SR (2004) Carbon and hydrogen isotopic fractionation during lipid biosynthesis in a higher plant (Cryptomeria japonica). Phytochemistry. doi: 10.1016/j.phytochem.2003.12.003**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cochrane MP, Duffus CM (1979) Morphology and ultrastructure of immature cereal grains in relation to transport. Ann Bot 44: 67–72**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci 27: 996-1001**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. J Exp Bot 55: 2447–2460**

- Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Coplen TB (1988) Normalization of oxygen and hydrogen isotope data. Chem Geol Isot Geosci Sect 72: 293–297**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Cormier M-A, Werner RA, Sauer PE, Gröcke DR, Leuenberger MC, Wieloch T, Schleucher J, Kahmen A (2018) 2H-fractionations during the biosynthesis of carbohydrates and lipids imprint a metabolic signal on the  $\delta^2\text{H}$  values of plant organic compounds. New Phytol 218: 479–491**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In E Tongiorgi, ed, Proc. a Conf. Stable Isot. Oceanogr. Stud. Palaeotemperatures. Lischi and Figli, Pisa, pp 9–130**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Cuntz M, Ogée J, Farquhar GD, Peylin P, Cernusak LA (2007) Modelling advection and diffusion of water isotopologues in leaves. Plant Cell Environ 30: 892–909**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Dansgaard W (1964) Stable isotopes in precipitation. Tellus 16: 436–468**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the "woody" tissues of plants: Implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. Geochim Cosmochim Acta 57: 3487–3492**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in Plant Ecology. Annu Rev Ecol Syst 33: 507–559**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Epstein S, Yapp CJ, Hall JH (1976) The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. Earth Planet Sci Lett 30: 241–251**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Epstein S, Thompson P, Yapp CJ (1977) Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198: 1209–15**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar G (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40: 503–537**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar GD, O'Leary MH, Berry JA (1982) on the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. Aust J Plant Physiol 9: 121–137**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar G, Richards R (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11: 539–552**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In A Ehleringer, J Hal, G Farquhar, eds, Stable Isot. Plant Carbon Water Relations, Academic P. San Diego, pp 47–70**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar G, Barbour MM, Henry BK (1998) Interpretation of oxygen isotope composition of leaf material. In HG Griffiths, ed, Stable Isot., Bios Scien. Oxford, pp 27–62**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar GD, Gan KS (2003) On the progressive enrichment of the oxygen isotopic composition of water along a leaf (Reprinted from Plant, Cell and Environment, vol 26, pg 801-819, 2003). Plant Cell Environ 26: 1579–1597**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Farquhar GD, Cernusak LA, Barnes B (2007) Heavy water fractionation during transpiration. Plant Physiol 143: 11–18**

- Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Feakins SJ, Sessions AL (2010) Controls on the D/H ratios of plant leaf waxes in an arid ecosystem. *Geochim Cosmochim Acta* 74: 2128–2141**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Ferrio JP, Mateo MA, Bort J, et al (2007) Relationships of grain  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  with wheat phenology and yield under water-limited conditions. *Ann Appl Biol* 150:207–215. doi: 10.1111/j.1744-7348.2007.00115.x**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Fischer RA, Rees D, Sayre KD, Lu Z-M, Condon AG, Saavedra AL (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci* 38: 1467–1475**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Pilot MS, Leuenberger M, Pazdur A, Boettger T (2006) Rapid online equilibration method to determine the D/H ratios of non-exchangeable hydrogen in cellulose. *Rapid Commun Mass Spectrom* 20: 3337–3344**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Foyer CH, Neukermans J, Queval G, Noctor G, Harbinson J (2012) Photosynthetic control of electron transport and the regulation of gene expression. *J Exp Bot* 63: 1637–1661**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Food and Agriculture Organization of the United Nations. 2017. Available at <http://www.fao.org/home/en/> (accessed 25 July 2017).**
- Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, Monson RK (2014) Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. *Tree Physiol* 34: 1–23**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gonfiantini R (1978) Standards for stable isotope measurements in natural compounds. *Nature* 271: 534–536**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gonfiantini R, Gratziu S, Tongiorgi E (1965) Oxygen isotopic composition of water in leaves. *Isot Radiat Soil Plant Nutr Stud. IAEA, Vienna*, pp 405–410**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gonfiantini MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Funct Plant Biol* 34: 83–94**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hayes JM (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Rev Mineral Geochemistry* 43: 225–277**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Helliker BR, Ehleringer JR (2000) Establishing a grassland signature in veins: O-18 in the leaf water of C3 and C4 grasses. *Proc Natl Acad Sci U S A* 97: 7894–7898**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Helliker BR, Ehleringer JR (2002) Differential  $^{18}\text{O}$  enrichment of leaf cellulose in C3 versus C4 grasses. *Funct Plant Biol* 29: 435–442**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hoganson CW, Babcock GT (1997) A metalloradical mechanism for the generation of oxygen from water in photosynthesis. *Science* 277: 1953–1956**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hou J, D'Andrea WJ, MacDonald D, Huang Y (2007) Hydrogen isotopic variability in leaf waxes among terrestrial and aquatic plants around Blood Pond, Massachusetts (USA). *Org Geochem* 38: 977–984**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hubick KT, Hammer GL, Farquhar GD, Wade LJ, von Caemmerer S, Henderson SA (1990) Carbon isotope discrimination varies genetically in C4 species. *Plant Physiol* 92: 534–537**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

Jensen A, Lorenzen B, Østergaard HS, Hvelplund EK (1990) Radiometric estimation of biomass and nitrogen content of barley grown at different nitrogen levels†. *Int J Remote Sens* 11: 1809–1820

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Jia S, Lv J, Jiang S, Liang T, Liu C, Jing Z (2015) Response of wheat ear photosynthesis and photosynthate carbon distribution to water deficit. *Photosynthetica* 53: 95–109

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Kahmen A, Schefuß E, Sachse D (2013) Leaf water deuterium enrichment shapes leaf wax n-alkane  $\delta D$  values of angiosperm plants I: Experimental evidence and mechanistic insights. *Geochim Cosmochim Acta* 111: 39–49

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Knoppik D, Selinger H, Ziegler-Jons A (1986) Differences between the flag leaf and the ear of a spring wheat cultivar (*Triticum aestivum* cv. Arkas) with respect to the CO<sub>2</sub> response of assimilation, respiration and stomatal conductance. *Physiol Plant* 68: 451–457

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Li X, Wang H, Li H, Zhang L, Teng N, Lin Q, Wang J, Kuang T, Li Z, Li B, et al (2006) Awns play a dominant role in carbohydrate production during the grain-filling stages in wheat (*Triticum aestivum*). *Physiol Plant* 127: 701–709

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Liu H, Guo B, Wei Y, Wei S, Ma Y, Zhang W (2015) Effects of region, genotype, harvest year and their interactions on  $\delta^{13}C$ ,  $\delta^{15}N$  and  $\delta D$  in wheat kernels. *Food Chem* 171: 56–61

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate change adaptation needs for food security in 2030. *Science* 319: 607–610

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lopes MS, Reynolds MP (2010) Dissecting drought adaptation into its phenotypic and genetic components in wheat. *Asp Appl Biol* 7–11

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Luo Y, Sternberg L (1991) Deuterium heterogeneity in starch and cellulose nitrate of cam and C<sub>3</sub> plants. *Phytochemistry* 30: 1095–1098

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Luo Y-H, Steinberg L, Suda S, Kumazawa S, Mitsui A (1991) Extremely Low D/H ratios of photoproduced hydrogen by cyanobacteria. *Plant Cell Physiol* 32: 897–900

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Martín-Gómez P, Barbeta A, Voltas J, Peñuelas J, Dennis K, Palacio S, Dawson TE, Ferrio JP (2015) Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? *New Phytol* 207: 914–927

Monneveux P, Reynolds MP, Trethowan R, González-Santoyo H, Peña RJ, Zapata F (2005) Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. *Eur J Agron* 22: 231–242

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Moore FC, Lobell DB (2015) The fingerprint of climate trends on European crop yields. *Proc Natl Acad Sci*. doi: 10.1073/pnas.1409606112

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Offermann C, Pedro Ferrio J, Holst J, Grote R, Siegwolf R, Kayler Z, Gessler A (2011) The long way down-are carbon and oxygen isotope signals in the tree ring uncoupled from canopy physiological processes? *Tree Physiol* 31: 1088–1102

Oweis T, Pala M, Ryan J (1998) Stabilizing rainfed wheat yields with supplemental irrigation and nitrogen in a mediterranean climate. *Agron J* 90: 672

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Pande P, Datta P, Bhattacharya S (1994) Biphasic enrichment of H<sub>2</sub><sup>18</sup>O in developing wheat grain water. *Indian J Exp Biol* 37: 30–31

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Pande P, Datta P, Bhattacharya S, Tyagi S (1995) Post-anthesis metabolic-enrichment of H<sub>2</sub><sup>18</sup>O in wheat grain. *Indian J Exp Biol* 33:

394–396

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Qi H, Coplen TB (2011) Investigation of preparation techniques for  $\delta^2\text{H}$  analysis of keratin materials and a proposed analytical protocol. *Rapid Commun Mass Spectrom* 25: 2209–2222**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant Growth Regul* 20: 157–166**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Sci* 42: 111–121**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Rieder S V., Rose IA (1959) The mechanism of the triosephosphate isomerase reaction. *J. Biol. Chem.* 234: 1007-1010**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Roden JS, Lin G, Ehleringer JR (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim Cosmochim Acta* 64: 21–35**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sachse D, Radke J, Gleixner G (2006)  $\delta\text{D}$  values of individual n-alkanes from terrestrial plants along a climatic gradient - Implications for the sedimentary biomarker record. *Org Geochem.* doi: 10.1016/j.orggeochem.2005.12.003**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sachse D, Billault I, Bowen GJ, Chikaraishi Y, Dawson TE, Feakins SJ, Freeman KH, Magill CR, McInerney FA, van der Meer MTJ, et al (2012) Molecular paleohydrology: Interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annu Rev Earth Planet Sci* 40: 221–249**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sadras VO (2004) Yield and water-use efficiency of water- and nitrogen-stressed wheat crops increase with degree of co-limitation. *Eur J Agron* 21: 455–464**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Salazar-Tortosa D, Castro J, Villar-Salvador P, et al (2018) The "isohydric trap": a proposed feedback between water shortage, stomatal regulation and nutrient acquisition drives differential growth and survival of European pines under climatic dryness. *Glob Chang Biol.* doi: 10.1111/gcb.14311**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sanchez-Bragado R, Elizab A, Zhou B, Serret MD, Bort J, Nieto-Taladriz MT, Araus JL (2014a) Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. *J Integr Plant Biol* 56: 444–454**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sánchez-Bragado R, Araus JL, Scheerer U, Cairns JE, Rennenberg H, Ferrio JP (2016) Factors preventing the performance of oxygen isotope ratios as indicators of grain yield in maize. *Planta.* doi: 10.1007/s00425-015-2411-4**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sauer PE, Schimmelmann A, Sessions AL, Topalov K (2009) Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. *Rapid Commun Mass Spectrom* 23: 949–956**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Schimmelmann A (1991) Determination of the concentration and stable isotopic composition of nonexchangeable hydrogen in organic matter. *Anal Chem.* doi: 10.1021/ac00021a013**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Schimmelmann A, Lawrence M, Michael E (2001) Stable isotopes ratios of organic H, C and N in the Miocene Monterey Formation, California. In CM Isaac, J Rullkötter, eds, *Monterey Form. From rocks to Mol.* Columbia University Press, pp 86–106**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)



**Schleucher J, Vanderveer P, Markley JL, Sharkey TD (1999) Intramolecular deuterium distributions reveal disequilibrium of chloroplast phosphoglucose isomerase. *Plant, Cell Environ.* doi: 10.1046/j.1365-3040.1999.00440.x**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Schmidt HL, Werner RA, Eisenreich W (2003) Systematics of 2H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochem Rev* 2: 61–85**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Schmidt HL, Robins RJ, Werner RA (2015) Multi-factorial in vivo stable isotope fractionation: causes, correlations, consequences and applications. *Isotopes Environ Health Stud.* doi: 10.1080/10256016.2015.1014355**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Schwendenmann L, Pendall E, Sanchez-Bragado R, Kunert N, Hölscher D (2015) Tree water uptake in a tropical plantation varying in tree diversity: interspecific differences, seasonal shifts and complementarity. *Ecohydrology* 8: 1–12**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Serret M., Ortiz-Monasterio I, Pardo A, Araus J. (2008) The effects of urea fertilisation and genotype on yield, nitrogen use efficiency,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in wheat. *Ann Appl Biol* 153: 243–257**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Shangguan ZP, Shao MA, Dyckmans J (2000) Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. *Environ Exp Bot* 44: 141–149**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sessions AL, Burgoyne TW, Schimmelmann A, Hayes JM (1999) Fractionation of hydrogen isotopes in lipid biosynthesis. *Org Geochem.* doi: 10.1016/S0146-6380(99)00094-7**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Smith FA, Freeman KH (2006) Influence of physiology and climate on  $\delta\text{D}$  of leaf wax n-alkanes from C3 and C4 grasses. *Geochim Cosmochim Acta.* doi: 10.1016/j.gca.2005.11.006**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Song X, Farquhar GD, Gessler A, Barbour MM (2014) Turnover time of the non-structural carbohydrate pool influences  $\delta^{18}\text{O}$  of leaf cellulose. *Plant Cell Environ.* doi: 10.1111/pce.12309**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sternberg L, Deniro MJ (1983) Isotopic Composition of Cellulose from C3, C4, and CAM plants growing near one another. *Science* 220: 947–9**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sternberg LO, Deniro MJ, Johnson HB (1984) Isotope ratios of cellulose from plants having different photosynthetic pathways. *Plant Physiol* 74: 557–561**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sternberg L, Deniro M, Savidge R (1986) Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. *Plant Physiol* 82: 423–427**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sternberg L (1988) D/H ratios of environmental water recorded by D/H ratios of plant lipids. *Nature* 333: 59–61**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Tambussi EA, Nogués S, Araus JL (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta* 221: 446–58**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Voltas J, Romagosa I, Lafarga A, Armesto AP, Araus JL, Sombbrero A (1999) Genotype by environment interaction for grain yield and carbon isotope discrimination of barley in Mediterranean Spain. *Aust J Agric Res* 50: 1263–1271**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Wassenaar LI, Hobson KA (2000) Improved method for determining the stable hydrogen isotopic composition ( $\delta\text{D}$ ) of complex organic**

materials of environmental interest. doi: 10.1021/ES990804I

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Winter K, Garcia M, Holtum JAM (2008) On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of Clusia, Kalanchoe, and Opuntia. J Exp Bot 59: 1829–40**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Winter K, Holtum JAM (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. J Exp Bot 65: 3425–41**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Williams DG, Coltrain JB, Lott M, English NB, Ehleringer JR (2005) Oxygen isotopes in cellulose identify source water for archaeological maize in the American Southwest. J Archaeol Sci 32: 931–939**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yakir D (1992) Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant, Cell Environ 15: 1005–1020**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yakir D, Deniro MJ (1990) Oxygen and hydrogen isotope fractionation during cellulose metabolism in Lemna gibba L. Plant Physiol 93: 325–332**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yakir D, Deniro MJ, Ephrath JE (1990a) Effects of water-stress on oxygen, hydrogen and carbon isotope ratios in 2 species of cotton plants. Plant Cell Environ 13: 949–955**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yakir D, Deniro MJ, Gat JR (1990b) Natural deuterium and oxygen-18 enrichment in leaf water of cotton plants grown under wet and dry conditions - evidence for water compartmentation and its dynamics. Plant Cell Environ 13: 49–56**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yang H, Leng Q (2009) Molecular hydrogen isotope analysis of living and fossil plants-Metasequoia as an example. Prog Nat Sci 19: 901–912**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yousfi S, Serret MD, Araus JL (2013) Comparative response of  $\delta^{13}C$ ,  $\delta^{18}O$  and  $\delta^{15}N$  in durum wheat exposed to salinity at the vegetative and reproductive stages. Plant Cell Environ 36: 1214–27**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14: 415–421**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Zhao LJ, Xiao HL, Liu XH (2007) Relationships between carbon isotope discrimination and yield of spring wheat under different water and nitrogen levels. J Plant Nutr 30: 947–963**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Zhou Y, Stuart-Williams H, Farquhar GD, Hocart CH (2010) The use of natural abundance stable isotopic ratios to indicate the presence of oxygen-containing chemical linkages between cellulose and lignin in plant cell walls. Phytochemistry. doi: 10.1016/j.phytochem.2010.03.001**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Zhou Y, Grice K, Chikaraishi Y, Stuart-Williams H, Farquhar GD, Ohkouchi N (2011) Temperature effect on leaf water deuterium enrichment and isotopic fractionation during leaf lipid biosynthesis: Results from controlled growth of C3 and C4 land plants. Phytochemistry. doi: 10.1016/j.phytochem.2010.10.022**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Zhou Y, Zhang B, Stuart-Williams H, Grice K, Hocart CH, Gessler A, Kayler ZE, Farquhar GD (2018) On the contributions of photorespiration and compartmentation to the contrasting intramolecular  $^2H$  profiles of C3 and C4 plant sugars. Phytochemistry. doi: 10.1016/j.phytochem.2017.11.004**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

✓ 

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**Ziegler H (1989) Stable Isotopes in Ecological research, Ecological. In: Rundel P, Ehleringer JR, Nagy K, eds. Berlin: Springer-Verlag.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Ziegler H, Osmond CB, Stichler W, Trimborn P (1976) Hydrogen isotope discrimination in higher plants: Correlations with photosynthetic pathway and environment. Planta 128: 85–92**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)