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*Seed Number and 100-Seed Weight of Pearl Millet (*Pennisetum glaucum* L.) Respond Differently to Low Soil Moisture in Genotypes Contrasting for Drought Tolerance*

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**Seed number and 100-seed weight of pearl millet
(*Pennisetum glaucum* (L.) R. Br.) respond differently to low
soil moisture in genotypes contrasting for drought tolerance**

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3 1 **Seed number and 100-seed weight of pearl millet (*Pennisetum glaucum L.*)**
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5 2 **respond differently to low soil moisture in genotypes contrasting for**
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7 3 **drought tolerance**
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37 17 **Abstract**
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39 18 Water stress after flowering, one of the major factors limiting yields of pearl millet, affects both
40 19 seed setting and grain filling, and is a consequence of more/less water used prior to anthesis.
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42 20 However, whether genotypes have different sensitivities for seed setting and filling under drought, if
43 21 exposed to similar stress intensity, is unclear. Experiments were conducted in two pairs of pearl millet
44 22 genotypes, i.e. PRLT2/89-33 and H77/833-2, 863B and 841B, contrasting for terminal drought
45 23 tolerance, and two genotypes, ICMR 01046 and ICMR 01029 (IL-QTLs) introgressed with a terminal
46 24 drought tolerance QTL from PRLT2/89-33 into H77/833-2. Total seed weight, panicle number, 100-
47 25 seed weight, seed number, and stover biomass were measured at different soil moistures, and
48 26 throughout-grain filling. Sensitive H77/833-2 had higher seed number and yield under well watered
49 27 (WW) conditions than in PRLT2/89-33 and IL-QTLs. Upon increases in water stress intensity,
50 28 H77/833-2 suffered losses mostly in stover biomass (45%) and seed number (60%) at 0.3 FTSW
51 29 whereas the biomass and seed number of PRLT2/89-33 decreased little (20% and 25%). The 100-seed
52 30 weight of H77/833-2 decreased only 20% under stress. Tolerant 863B also maintained a higher seed
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3 31 number and biomass under water stress than 841B. Seed growth duration in PRLT2/89-33 and IL-
4 32 QTLs was similar to that of H77/833-2 under WW conditions but lasted longer than in H77/833-2
5 33 under water stress (WS). Similarly, seed growth of 863B was longer than 841B under WS. It is
6 34 concluded that the higher seed yield of tolerant parents PRLT2/89-33 and 863B, and of IL-QTLs
7 35 under WS was explained by the retention of a higher number of seeds than in sensitive lines, while the
8 36 decrease in the 100-seed weight was proportionally less than the decrease in seed number. Phenotype
9 37 with lesser number and larger size of panicles and larger grain size, like genotypes PRLT2/89-33 and
10 38 863B, withstood post-anthesis water stress better. IL-QTL inherited part of these characteristics,
11 39 indicating a role for the terminal drought QTL in maintaining larger seed number and higher 100-seed
12 40 weight. The continuous stover biomass increase under WW in H77/833-2, due to tillering, might
13 41 indicate that tiller growth and grains are in competition for resources after anthesis and this may relate
14 42 to the relatively shorter grain filling period.
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23 44 **Key words:** Post flowering water stress, carbohydrates, transpiration, biomass, yield
24 45 components
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30 48 **Introduction**

31 49 Pearl millet is widely grown in the arid zone of northwestern India and also in the
32 50 Sahelian zone where there is no alternative. Drought stress is a regular feature in these
33 51 environments, occurring at unpredictable time and intensity (Sharma and Pareek, 1993; van
34 52 Oosterom et al., 1996), but being most common during grain filling. Successful grain filling
35 53 is therefore one of the criteria in selecting the genotypes for improved adaptation to stress.
36 54 Farmers preferentially grow high tillering landraces particularly when drought stress is highly
37 55 unpredictable as in the case of arid areas of western Rajasthan (Van Oosterom et al., 1996).
38 56 High tillering genotypes are associated with small sized panicles and low individual grain
39 57 mass, which can be further decreased if the grain filling ability is impaired by water stress.
40 58 However, genotypes with this type of development pattern are better able to cope with
41 59 unpredictable stress because of their better capacity to compensate for the failure of the main
42 60 panicle than low tillering large sized panicles (Bidinger and Hash, 2004). By contrast,
43 61 genotypes with large grain size and low tillering have been widely adopted (Kelley et al.,
44 62 1996; van Oosterom et al., 1996) in the wetter eastern areas of Rajasthan where pre-flowering
45 63 drought stress is unlikely to occur but post-anthesis drought is predominant. The question of
46 64 grain size under stress conditions is also important to address for grain quality since larger
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3 65 seeds have a higher flour yield (Rooney and McDonough, 1987) and a stress effect on grain
4 66 size could decrease flour yields.
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6 67 One of the unanswered questions is whether these different grain types are differently
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8 68 affected by water stress. In pearl millet grain yield is highly correlated with grain number
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10 69 (Bidinger and Raju, 2000). Large grain number correlates with small individual grain mass
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12 70 and short grain filling periods, and is an important adaptive feature of pearl millet to the arid
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14 71 climates (DeWet et al., 1992). In contrast, large grain size is a highly preferred characteristic
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16 72 according to farmer survey, allowing higher market price (Phul and Athwal, 1969, IARI
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18 73 report in magazine- National Herald 2006). Large grain mass also confers faster rates of
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20 74 seedling emergence, faster initial seedling and early crop growth (Lawan et al., 1985, Siband
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22 75 et al., 1978, Chhina and Phul, 1982), improved processing quality of the grain, easy
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24 76 decortications, and better flour yield with both commercial milling and hand-pounding
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26 77 milling methods (Rooney and McDonough, 1987). Whether different grain types are
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28 78 differently affected by terminal stress is not known in pearl millet.

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30 79 A major drought QTL on linkage group two (LG2) explaining 23% of variation in grain
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32 80 yield under severe drought environments was identified (Yadav et al., 2002; Yadav et al.
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34 81 2004; Bidinger et al 2007), and accounted for a better seed set and a better grain filling. This,
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36 82 in turn, was explained by a conservative water use when water was non-limiting, which made
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38 83 more water remains available for grain filling (Kholová et al., 2010a, 2010b; Vadez et al.,
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40 84 2013). Since the QTL is responsible for differences in grain filling, another possible
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42 85 explanation for the difference between tolerant and sensitive lines could be differences in the
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44 86 soil moisture thresholds where grain filling stops. Such information is not available in pearl
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46 87 millet and the existence of lines (ILs) introgressed with a major terminal drought tolerance
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48 88 QTL allows this exploration. Here, we address this question by following grain filling in
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50 89 different soil moisture conditions. Our hypothesis is that grain filling may stop at different
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52 90 levels of soil moisture in different genotypes which could explain part of the grain yield
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54 91 differences under terminal drought conditions.

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56 92 The first objective of this study was to evaluate whether the response of yield
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58 93 components to drought stress differed between tolerant and sensitive genotypes, which was
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60 94 done by measuring grain yield, grain number, grain size, and stover biomass under different
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96 95 levels of soil moisture. The second objective was to assess how these different components
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98 96 evolved during the grain filling period in different genotypes, and this was done by sequential
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100 97 harvests during grain filling and until maturity. The work was carried out using contrasting

98 genotypes, including ILs containing a terminal drought tolerance QTL in order to assess
99 whether mechanisms related to differences in seed filling are underlying this QTL.

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102 **Materials and methods:**

103 **Plant material**

104 Parental genotypes: Pearl millet genotypes differing in terminal drought tolerance were
105 selected. Two pairs of parents PRLT2/89-33 (tolerant) and H77/833-2 (sensitive), ICMB 863-
106 P2 (tolerant, then referred to as 863B) and 841B-P3 (sensitive, then referred to as 841B)
107 selected for the study contrasted for seed yield under terminal drought conditions based on
108 previous experiments (Yadav et al., 2002; Serraj et al., 2005). Both pairs of parental lines
109 were tested in that study because a terminal drought tolerance QTL was identified in both
110 derived mapping populations. Tolerance/sensitivity was assessed using testcross hybrids of
111 these parental inbred lines, using 843A and H77/833-2A as a male sterile tester for each pair
112 respectively. PRLT2/89-33 is a low tillering, large panicle experimental line (Andrews and
113 Anand Kumar, 1996) and H77/833-2 is a high tillering line with small panicles (Kapoor et al.,
114 1989). More details describing the two parental pairs can be found in Kholová et al., (2010a).

115 Introgression lines (IL-QTLs): QTL introgression lines were developed in the background of
116 sensitive parent H77/833-2 (recurrent parent) by introgressing the QTL from drought tolerant
117 donor parent PRLT2/89-33 (QTL identified on LG2 by Yadav et al., 2002; Yadav et al. 2004;
118 Bidinger et al., 2007) and the resulting F1 was backcrossed to the recurrent parent H77/833-2
119 for four generations (more details in Kholová et al., 2010a). IL-QTLs in H77/833-2
120 background (ICMR01029 and ICMR01046) were also tested as test-cross hybrids using 843A
121 as a male sterile tester.

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123 **Assessment of grain filling and seed number under different levels of soil moisture**

124 Experiment 1 (Exp.1) was designed to assess the response of transpiration and of agronomic
125 characteristics, including grain size and grain number, to different levels of moisture stress, in
126 two parental pairs; i.e. PRLT 2/89-33 and H77/833-2, 863B and 841B . Plants were grown in
127 pots filled with 9.5 kg of a mixture of Alfisol, sand, and manure (5:2:1) under glass house
128 conditions with 17.6°C min and 35.5°C max temperature and 40-75% relative humidity (RH).
129 Growth in the pots was very satisfactory and plant height was similar to the field conditions.
130 Although small pot size could have affected the root/shoot ratio, these growth conditions

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3 131 were unlikely to have affected genotypic differences in response to drought. Five water
4 132 treatments were used: One well-watered control and four water stress treatments in which the
5 133 soil moisture content was re-adjusted daily to a constant value of 50%, 40%, 30%, and 20%
6 134 of the fraction of transpirable soil water (i.e. 0.5, 0.4, 0.3, 0.2 FTSW) by deducting 890,
7 135 1070, 1250 and 1420g of water, respectively, from the pots maintained at field capacity. This
8 136 was based on previous experiments in which it was shown that, when exposed to a
9 137 progressive water stress: (i) genotypes did not vary in the amount of water they could extract
10 138 for transpiration from a given soil weight; (ii) there was approximately 180g of transpirable
11 139 water per kg of soil. These FTSW values reflected an average for the entire pot and it does
12 140 not exclude the possibility that, at re-watering, the top part of the pot would have been wet
13 141 while the remaining part of the pot would have been dry. We believe this was not an issue for
14 142 the plant response. First, this would be a similar situation in nature. Second, the most limiting
15 143 factor for the plant was the limited amount of water that was received every day and this was
16 144 relatively similar for each genotype. The experimental design was a complete block design
17 145 with water treatment as main block and genotypes as sub-factor in each main block and
18 146 randomized five times.

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29 147 Plants were grown under fully irrigated conditions until panicle emergence. As soon
30 148 as panicles were emerged all the pots were watered and allowed to drain overnight to reach
31 149 field capacity. The following morning, pots were wrapped with plastic bags around the base
32 150 of the stem to cease soil evaporation and pots were subsequently weighed. Pots from all
33 151 treatments were maintained under well-watered (WW) conditions by daily re-watering the
34 152 pots up to 80% field capacity (0.8 FTSW) until flowering. Water stress treatments were
35 153 imposed from flowering time onwards by gradually decreasing the water level to 0.5, 0.4, 0.3,
36 154 and 0.2 FTSW. All genotypes flowered within about two to three days from one another, so
37 155 that there was only a minimum time difference with the time when water stress was initiated.
38 156 The FTSW represents how much water is available for transpiration in the pot, as a
39 157 proportion of what is available at field capacity (1, or 100%). In order to impose a gradual
40 158 stress, the desired soil moisture levels were reached only 4-5 days after flowering. The
41 159 purpose of this was also to ensure that reproduction would take place under WW conditions
42 160 and before the stress levels were imposed. The pot weights were maintained at these set
43 161 levels of FTSW until maturity (between 30 and 38 days after flowering), by daily weighing
44 162 and re-watering to set target pot weight. Harvested plants were oven-dried in a forced-air
45 163 oven at 70°C for three days. Stover biomass, panicle number, total seed weight, seed number,
46 164 and 100- seed weight were then measured.

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3 1654 166 **Dynamics of grain filling**

5 167 Experiment 2 and 3 (Exp.2 and Exp.3) followed up how grain yield, grain number, grain size,
6 168 stover biomass, and transpiration evolved during the grain filling period in plants exposed to
7 169 two water regimes: (i) a well-watered control (WW); (ii) a soil moisture content of 0.3 FTSW
8 170 (with 30% moisture) from four days after flowering and until maturity. Exp.2 included ICMR
9 171 01029 and ICMR 01046, PRLT2/89-33 and H77/833-2. Exp.3 included 863B and 841B. The
10 172 transpiration values, obtained from daily weighing, were normalized against control plants to
11 173 get normalized transpiration ratio. For that, the transpiration value of each replicate was
12 174 divided by the average transpiration of WW plant to get a transpiration ratio. A second
13 175 normalization was carried out to take care of plant to plant variation in size, by dividing the
14 176 transpiration ratio by the mean transpiration ratio value of the first three days of the
15 177 experiment, before the occurrence of any stress. Sequential harvests were carried out at 10,
16 178 20, and 30 days after flowering, the last harvest corresponding to maturity. The experimental
17 179 design was a complete block design with the three sequential harvests as main blocks, water
18 180 regimes as sub-blocks, and genotypes as sub-factor in each main sub-block and randomized
19 181 five times. Plants were grown under same conditions. Treatment imposition and harvest
20 182 procedures followed those of Exp.1.

21 183 Extraction and determination of total carbohydrates was done from the penultimate
22 184 internodes (below the peduncle) harvested at maturity, by grinding 200mg of fresh tissue
23 185 twice with 70% ethanol at 90°C. These ethanol extracts were pooled, centrifuged at 10,000 g
24 186 for 10min and the supernatant was taken for estimation. Total sugars were estimated by
25 187 Dubois et al. (1956) method using phenol and sulphuric acid.

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27 189 **Statistical analysis:**

28 190 One way ANOVA was carried out for genotypic differences within the treatment. ANOVA
29 191 was done with the statistical program package CoStat version 6.204 (Cohort Software,
30 192 Monterey, CA, USA). Grouping of the genotypes in between the treatments was done using
31 193 Duncan's multiple range tests through the statistical program SAS version 9.2 to compare the
32 194 treatment effect from Exp.1. For Exp.2 and Exp.3, Duncan's multiple range tests through
33 195 SAS version 9.2 was used to compare the genotypes at different times of harvests separately
34 196 for control and stress during the grain filling period. ANOVA was carried out for genotypic
35 197 differences in carbohydrates separately under WW and water stress.

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200 **Results:**201 **Grain yield response to varying water stress treatments**

202 Seed yield decreased significantly at 0.4 FTSW compared to the WW treatment, and then
203 further decreased significantly at 0.3 and 0.2 FTSW in both the parental pairs ($P < 0.0001$ Fig.
204 1a and 1b, Exp.1). However, genotypic differences in total seed weight were observed under
205 WW conditions and 0.3 FTSW in both pairs. There were significant genotype-by-treatment
206 interactions for the seed yield ($P < 0.02$), so that under WW conditions, H77/833-2 had
207 significantly higher total seed weight than PRLT2/89-33 ($P < 0.1$), whereas at 0.3 and 0.2
208 FTSW, PRLT2/89-33 had higher total seed weight than H77/833-2 ($P < 0.1$ Fig. 1a, Exp.1).
209 With the parental pair 863B and 841B (Exp.1, Fig. 1b), genotype 863B (tolerant) had
210 significantly higher total seed weight than 841B (sensitive) under WW conditions ($P < 0.01$)
211 and water stress treatments 0.4 and 0.3 FTSW ($P < 0.1$).

212 The evolution of seed weight over time after flowering at 0.3 FTSW and under WW
213 conditions was followed in Exp.2 (Fig. 1c). Under WW conditions, the seed weight of
214 H77/833-2 significantly increased in all sequential harvests ($P < 0.0001$), with the highest
215 seed yield ($P < 0.05$) at maturity, whereas in PRLT2/89-33, ICMR 01046, and ICMHR 01029
216 the total seed weight did not increase significantly beyond 20 days after flowering. Under WS
217 conditions (0.3 FTSW), the increase in seed weight was somewhat slower, with a gradual
218 increase in total seed weight. All genotypes attained their maximum seed weight at 20 days
219 after flowering except ICMR 01046 that reached its highest total seed weight at 30 days after
220 flowering (Fig. 1c). PRLT2/89-33, ICMR 01029 and ICMR 01046 had significantly higher
221 total seed weight than H77/833-2 at maturity ($P < 0.05$), in agreement with Exp. 1. Under
222 WW conditions in the parental pair 863B and 841B (Exp.3, Fig: 1d), the total seed weight
223 increased significantly until the last harvest ($P < 0.05$). Under WS conditions, both the
224 parental lines showed maximum seed weight at 20 days after flowering (Fig. 1d). There,
225 863B (tolerant) had significantly higher seed weight than 841B (sensitive) under WW and
226 WS conditions in agreement with Exp.1 ($P < 0.05$).

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228 **Panicle number, seed number and 100-seed weight response to water stress**

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3 229 Water stress strongly decreased panicle number, especially in the high tillering types. Indeed,
4 230 panicle number decreased at treatment 0.3 FTSW ($P < 0.0001$, Exp.1, Fig.2a) and these
5 231 changes were driven by a sharp decrease in panicle number of high tillering H77/833-2
6 232 (sensitive parent, ($P < 0.01$), reflecting significant genotype-by-treatment interaction (P
7 233 < 0.05). In the parental pair 863B and 841B (Exp.1, Fig.2b) significant treatment differences
8 234 were observed at 0.4 FTSW with no further decrease at more severe treatment ($P < 0.0001$).
9 235 Similar to the above parental pair 841B (sensitive parent) had significantly higher number of
10 236 panicles in all the treatment except at 0.3 FTSW ($P < 0.01$).

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17 237 The seed number of the parental pair PRLT2/89-33 and H77/833-2 decreased
18 238 significantly at 0.4 FTSW although this decrease affected only H77/833-2 compared to WW
19 239 conditions. The seed number of the parental pair then further decreased at 0.2 FTSW (P
20 240 < 0.0001) and seed number decreased in both genotypes compared to 0.4 FTSW (Exp.1, Fig.
21 241 3a). The genotype-by-treatment interaction for seed number was also highly significant (P
22 242 < 0.001). H77/833-2 (sensitive parent) had significantly higher seed number than PRLT2/89-
23 243 33 (tolerant parent) under WW, 0.5 FTSW, and 0.4 FTSW ($P < 0.01$) but both genotypes had
24 244 similar seed number at 0.3 and 0.2 FTSW. At these FTSW levels, the seed number of
25 245 H77/833-2 was 65% less than the WW conditions. In the parental pair 863B and 841B
26 246 (Exp.1, Fig. 3b), the seed number decreased significantly at 0.4 FTSW compared to WW
27 247 conditions, with a further significant decrease at 0.3 and then 0.2 FTSW ($P < 0.0001$).
28 248 Genotypes 863B and 841B had similar seed number at all FSTW levels except 0.3 ($P < 0.05$).

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37 249 In Exp.2 (Fig. 3c) the evolution in the seed number over time after flowering was
38 250 observed. Under WW conditions the seed number remained similar in PRLT2/89-33
39 251 throughout the grain filling period. By contrast, the seed number increased throughout the
40 252 seed filling period in the other genotypes. In ICMR 01029 the maximum seed number was
41 253 observed at 20 days after flowering whereas ICMR 01046 and H77/833-2 reached the highest
42 254 seed number at 30 days. This was likely related to the development of reproductive tillers in
43 255 these three high tillering types. Under WS conditions the seed number of PRLT2/89-33 and
44 256 ICMR 01046 remained similar across the different harvests. By contrast, the seed number
45 257 increased in H77/833-2 and ICMR 01029 and the highest seed number was reached at 20
46 258 days and 30 after flowering respectively. However, even at its highest seed number (20 days
47 259 after flowering) the seed number of H77/833-2 had decreased drastically compared to the
48 260 WW treatment while it did decrease relatively less in the other genotypes, in agreement with
49 261 Exp.1. In the parental pair 863B and 841B under WW conditions the maximum seed number
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3 262 was reached at the last harvest i.e., 30 days after flowering in both 863B (tolerant) and 841B
4 263 (sensitive) (Exp.3, Fig. 3d). Under water stress, there was no further significant increase in
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6 264 seed number in both genotypes beyond 20 days after flowering.

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8 265 The 100-seed weight started to decrease at lower soil moisture levels than biomass
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10 266 and seed yield. In the PRLT2/89-33 and H77/833-2 pair (Exp.1, Fig. 4a), there was no
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12 267 significant treatment effect on the 100-seed weight until FTSW was down to 0.2. The
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14 268 genotype-by-treatment interaction for the 100-seed weight was also highly significant (P
15 269 <0.004). The 100-seed weight decreased gradually in PRLT2/89-33 from 0.5 to 0.3 FTSW,
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17 270 and the 100-seed weight of PRLT2/89-33 was about 23% lower at 0.3 FTSW than under WW
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19 271 conditions, with a rapid drop between 0.3 and 0.2 FTSW. By contrast, in H77/833-2 there
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21 272 was no change in the 100-seed weight between the WW control and 0.3 FTSW, but there was
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23 273 a rapid drop at 0.2 FTSW. Therefore, PRLT2/89-33 (tolerant parent) had significantly higher
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25 274 100-seed weight than H77/833-2 (sensitive) until FTSW was down to 0.3 ($P <0.001$ at WW
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27 275 and $P <0.05$ at 0.3 FTSW) but both the genotypes had similar low 100-seed weights at 0.2
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29 276 FTSW. In the parental pair 863B and 841B the 100-seed weight decreased significantly at 0.3
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31 277 FTSW compared to the WW treatment, and then further decreased at 0.2 FTSW ($P <0.0001$,
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33 278 Exp.1, Fig. 4b). Genotypic differences were significant until FTSW was down to 0.4 with
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35 279 863B parent having a significantly higher seed weight than 841B ($P <0.01$). At 0.3 and 0.2
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37 280 FTSW the 100-seed weight was not significantly different between the two genotypes.

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39 281 The evolution of the 100-seed weight was followed at 0.3 FTSW and under WW
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41 282 conditions in the post-flowering and grain filling period. Under WW conditions all the
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43 283 genotypes attained the maximum 100-seed weight (used as a proxy for seed filling) at 20
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45 284 days after flowering and there was no further significant increase at 30 days after flowering.
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47 285 Under water stress conditions the increase in 100-seed weight was more gradual. For instance
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49 286 PRLT2/89-33, ICMR 01046, and ICMR 01029 reached their maximum 100-seed weight at
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51 287 30 days after flowering. By contrast, the 100-seed weight did not change in H77/833-2 across
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53 288 the different harvests (Exp. 2; Fig. 4c). Unlike Exp.1, the 100-seed weight of H77/833-2
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55 289 decreased at 0.3 FTSW compared to the WW conditions. However, in both experiments it
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57 290 was the large seed number decreased that affected the seed yield of H77/833-2. In the
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59 291 parental pair 863B and 841B under both WW and WS conditions both genotypes 863B and
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292 841B reached their maximum 100-seed weight at 20 days after flowering (Exp. 3; Fig. 4d).

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3 294 **Stover biomass, transpiration and total soluble sugars response to water stress**

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5 295 Stover biomass decreased gradually with increase in the water stress treatment (Exp.1, Fig.
6 296 5a). In the parental pair PRLT2/89-33 and H77/833-2 it was not until 0.3 FTSW that a
7 297 significant decrease in biomass was observed ($P < 0.0001$). However, the genotype-by-
8 298 treatment interaction for stover biomass was not significant. Within treatment, significantly
9 299 higher biomass was observed in PRLT2/89-33 (tolerant) than H77/833-2 (susceptible) at 0.3
10 300 FTSW only ($P < 0.05$, Fig. 5a). In the parental pair 863B and 841B (Exp. 1, Fig. 5b) stover
11 301 biomass decreased significantly compared to the WW control at 0.3 and 0.2 FTSW (P
12 302 < 0.0001). Within treatments 863B (tolerant) had significantly higher biomass than 841B
13 303 (sensitive) at 0.3 and 0.2 FTSW treatments ($P < 0.05$, Fig. 5b).

14
15 304 The evolution of stover biomass over time after flowering was followed at 0.3 FTSW
16 305 and under WW conditions at different timings after flowering. Under WW conditions there
17 306 was no increase in the vegetative biomass across the three different harvests in PRLT2/89-33
18 307 (Exp. 2; Fig. 5c). By contrast, in the other three lines stover biomass increased between
19 308 flowering and maturity. In H77/833-2 the highest accumulation of stover biomass was
20 309 observed at the last harvest (30 days after flowering) while in ICMR 01046 and ICMR 01029,
21 310 there was no significant increase in stover biomass beyond 20 days after flowering (Fig. 5c).
22 311 Under WS conditions none of the genotypes showed any significant increase in stover
23 312 biomass after flowering (Fig. 5c) but at maturity PRLT2/89-33 (tolerant) had significantly
24 313 higher biomass than H77/833-2 (sensitive), in agreement with Exp.1 ($P < 0.05$). In the
25 314 parental pair 841B and 863B there was no increase in the stover biomass after flowering
26 315 irrespective of the time of harvest in any of the water treatment (Fig. 5d). Under WS
27 316 conditions, 863B had significantly higher biomass than 841B in agreement with Exp.1 (P
28 317 < 0.05).

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30 318 The transpiration of the WW plants was higher than in the different FTSW treatment
31 319 although the difference with the 0.5 FTSW was small. Within the WW (well watered) and the
32 320 different water stress treatments (0.5, 0.4, 0.3, 0.2 FTSW) of Experiment 1 (Fig. 6a) there
33 321 were only slight differences between tolerant and sensitive genotypes. However, after
34 322 normalizing the transpiration data for each individual genotype tolerant parent PRLT2/89-33
35 323 had lower NTR (normalized transpiration ratio) than the sensitive parent H77/833-2 at 0.3
36 324 FTSW (Fig. 6b). Similar results were observed in the case of 863B and 841B parental pair
37 325 where the difference in transpiration showed no clear trend (Fig. 6c) but where 863B (tolerant
38 326 parent) had lower NTR than 841B (sensitive parent) at 0.3 and 0.5 FTSW (Fig. 6d). In Exp.2,
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3 327 transpiration (Supplementary Fig. 1a) was similar in PRLT 2/89-33, H77/833-2, ICMR
4 328 01046, and ICMR 01029 but the NTR of tolerant parent PRLT2/89-33 and ICMR 01029 was
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6 329 lower than the sensitive parent H77/833-2 and ICMR 01046 (Supplementary Fig. 1b). Similar
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8 330 results were found in Exp.3, where transpiration (Supplementary Fig. 1c) was similar in both
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10 331 the genotypes but NTR (Supplementary Fig. 1d) of tolerant parent 863B was lower than
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12 332 sensitive parent 841B in the middle of the grain filling period. This trend of lower NTR was
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14 333 also observed in Experiment 1 (Fig. 6d) although it was not significant then.

15 334 Under WW conditions and water stress in Experiment 2 and 3 (Fig. 7), soluble sugars
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17 335 differed significantly among the genotypes. Soluble sugars decreased dramatically under WS
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19 336 in PRLT2/89-33, ICMR 01029 and 863B, whereas it did not decrease in H77/833-2, ICMR
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21 337 01046 and 841B. The soluble sugars were expressed per unit of fresh weight. We do not
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23 338 expect the stem relative water content to vary much between genotypes under WS at 30 days
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25 339 after flowering (i.e. their absolute transpiration was similar across genotypes), so that
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27 340 genotypic differences under WS conditions would likely not change if data were expressed
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29 341 per unit of dry weight.

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32 343 **Discussion**

33 344 **Grain yield decrease under drought was due to a greater effect on seed number than on** 34 35 345 **seed size**

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37 346 The higher seed yield of sensitive parent H77/833-2 under WW conditions was explained by
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39 347 its higher number of productive tillers (Fig. 2a) which would also explain why seed yield
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41 348 increased between the harvest at 20 days after flowering and the harvest at maturity (Fig. 1c).
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43 349 Under WS conditions the large seed yield decrease of H77/833-2 was then mostly due to a
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45 350 decrease in seed number, which was in part explained by a decreased number of panicles
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47 351 whereas in PRLT2/89-33 the yield decrease was related to a decrease in the 100-seed weight.
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49 352 However, the reduction of 100-seed weight in PRLT2/89-33 was proportionally less (23% at
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51 353 0.3 FTSW compared to the WW conditions, Fig. 4a) than the reduction of seed number in
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53 354 H77/833-2 (about 65% lower at 0.3 FTSW than under the WW conditions, Fig. 3a). This
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55 355 explained the lower seed yield of H77/833-2 than PRLT2/89-33 under WS conditions.
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57 356 Therefore, the lower seed yield in H77/833-2 was due to combined effect of a reduction in
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59 357 panicle number (productive tillers) and a drastic reduction in seed number. The reduced seed
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358 number might itself result from a combination of decreased number of panicles bearing seeds

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3 359 and abortion of some of the grain after flowering, although we don't have sufficient data to
4 360 fully conclude on this. Therefore, future work should look at each individual tiller to assess
5 361 the decrease in seed number and seed size in each of these, in relation to the timing of
6 362 flowering of these tillers. An interesting insight would then be to compare sink strength in
7 363 each tiller and carbohydrate content. In the 841B and 863B pair the number of seeds also
8 364 decreased relatively more in 841B than in 863B at 0.3 FTSW and decrease yield more in
9 365 841B. These results are in agreement with Blum et al. (1990) who suggested that yield
10 366 reduction under water stress at later stages was mainly due to the number of grains per spike.
11 367 These data agree with earlier work in pearl millet (eg Bidinger et al., 1987; 2004). Similar
12 368 results were reported by Izanloo et al. (2008) in wheat who reported that water stress reduced
13 369 yield through tiller abortion and lower grain number per spike. Interestingly, similar
14 370 observations have been made in legumes, i.e. in bean (Szilagyi., 2003), and chickpea
15 371 (Zaman-Allah et al., 2011), where the reduction in seed yield under water stress was due to a
16 372 decrease in pod number per plant and in seed number per pod, but not to a reduction in the
17 373 100-seed weight.

18 374 The higher seed yield of PRLT2/89-33 under WS conditions was then due to its capacity to
19 375 retain seed number relatively unchanged at 0.3 FTSW (about 75% of that under WW
20 376 conditions) and to limit the reduction in seed size. This may be related to the fact that the
21 377 increase in grain size is limited in high tillering genotype H77/833-2 either due to genetically
22 378 maximum grain size or inadequate availability of assimilates for grain filling. Response of
23 379 stronger sink towards higher yields has been reported in barley and wheat (Voltas et al.,
24 380 1997; Cartelle et al., 2006). Similarly, the low-tillering large seeded genotype PRLT2/89-33
25 381 have greater ability to adjust grain number and individual grain mass thus affecting panicle
26 382 productivity (Bidinger and Raju, 2000). These genotypes have been bred for higher yield
27 383 through maintaining higher individual grain mass.

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385 **Traits dynamics in parental and introgression lines**

386 Under well watered conditions, in PRLT2/89-33, there was no increase in vegetative
387 biomass after flowering whereas the vegetative biomass of H77/833-2, but also that of
388 introgressed lines having drought QTL of tolerant donor parent PRLT2/89-33, increased
389 during grain filling. This increase in both vegetative biomass and in grain yield in the high
390 tillering material was also possible because of the fairly wide spacing of the plants in the

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3 391 glasshouse (about 5 plant m⁻²). Under water stress (0.3 FTSW), a significant decrease in the
4 392 vegetative biomass of high tillering H77/833-2 genotype was likely related to a decrease in
5 393 the number of productive tillers, in part shown in Fig. 2a. Therefore, this led to a significantly
6 394 lower yield than in PRLT2/89-33 and ILs. The advantage conferred by the introgression of
7 395 the QTL over H77/833-2 was in two ways: (i) larger seed size than in H77/833-2 and the
8 396 capacity to sustain seed filling well into the drought period, whereas the seed filling duration
9 397 of H77/833-2 was short; (ii) having a seed number intermediate between the two parents and
10 398 the capacity to retain a relatively high seed number under WS conditions. These experimental
11 399 results are in agreement with previous findings (Serraj et al., 2005). Another advantage of the
12 400 IL lines could have been in having larger panicle size than the recurrent parent, although we
13 401 have no data to support this and only qualitative observations. Similar conclusions have also
14 402 been drawn by Bolanos (1995) where superior yield of hybrids was mostly due to larger sink
15 403 (larger ear weight and ear growth rate). PRLT2/89-33 and ILs had indeed a high grain filling
16 404 ability under both WW and WS conditions, shown by their capacity to sustain seed filling
17 405 until maturity, whereas the seed size of H77/833-2 was the same at each of the three harvests
18 406 under WS conditions (Fig. 4c). ICMR 1046 maintained the 100-seed weight similar to
19 407 drought tolerant PRLT2/89-33 parent under WS conditions. This provides us evidence that
20 408 the donor QTL help in maintaining the grain filling ability. The seed number in ILs was also
21 409 decreased less dramatically than in recurrent parent H77/833-2. Among the two introgression
22 410 lines that are nearly isogenic, ICMR 01046 and ICMR 01029 were highly similar to
23 411 PRLT2/89-33 parent for most traits. Thus, besides a large grain size with low grain number to
24 412 keep up the yield under post flowering drought stress (Bidinger and Raju, 2000), as shown in
25 413 the previous section, the added advantage of tolerant materials seems to be in the capacity to
26 414 maintain grain filling for a longer period. This was not related to a higher water extraction
27 415 capacity, but rather from having water saving mechanisms operating earlier in the crop cycle
28 416 and making water available during the grain filling period (Kholova et al., 2010a; Vadez et
29 417 al., 2013).

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419 **Carbohydrate translocation and transpiration**

420 Synthesis, storage and mobilization of carbohydrates under water stress are essential
421 processes for grain filling (Gupta et al., 2011). Reduction of assimilates under water stress
422 that limits grain filling has been reported (Mahalakshmi et al., 1993). The percentage
423 decrease in total soluble sugars was high in ICMR 01029 (52%) and tolerant parents

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3 424 PRLT2/89-33 (24%), 863B (25%). However, there was no reduction under WS conditions in
4 425 sensitive parents H77/833-2 and 841B (Fig.7). Therefore, we may interpret that part of the
5 426 seed setting or seed filling failure was related to the inability to remobilize sugars from the
6 427 stem in sensitive lines. The reasons for that are unknown but could be related to poor
7 428 translocation or enzymatic activities, and further research would be needed in the
8 429 mechanisms that regulate grain filling.

13 430 Transpiration, which was used as a simple proxy for photosynthesis, decreased with
14 431 progressive exposure to water stress. The absolute transpiration values were not very
15 432 different between genotypes at any of the treatment although relative to the control, the NTR
16 433 of tolerant genotypes was below that of the sensitive parents. Interestingly, once the FTSW
17 434 was set at each of the pre-determined levels, transpiration remained relatively constant and
18 435 did not vary much between genotypes. Since the vegetative biomass did not increase in
19 436 PRLT2/89-33 during grain filling under WW conditions, and it increased only slightly in ILs,
20 437 the transpiration occurring during the grain filling period would have mostly supported the
21 438 filling of grains. By contrast, in H77/833-2, the large increase in vegetative biomass during
22 439 the grain filling period under WW implies that tiller growth continued well into the grain
23 440 filling period and then contributed to grain yield, if water was available. Under water stress,
24 441 although there was no significant increase in vegetative biomass, we may hypothesize that
25 442 competition may have occurred between grain filling of the early tillers and the developing
26 443 tillers, which may in part explain the failure of a number of grains, but also an inadequate
27 444 carbon accumulation that limits tiller growth and then limits yield under WS. In the case of
28 445 863B, the higher transpiration efficiency of this line, reported earlier (Kholová et al., 2010b),
29 446 might have also contributed to the higher vegetative biomass than in 841B under 0.3 FTSW,
30 447 and therefore a higher yield. Therefore, higher yield of 863B parent was apparently due to its
31 448 combined effort in keeping up the number of reproductive tillers and seed number in
32 449 comparison to the sensitive parent 841B which had lower vegetative biomass (likely linked to
33 450 lesser number of reproductive tillers, Fig.2b) and seed number under water stress (0.3
34 451 FTSW). This data is in support with previous results on these two lines (Yadav et al., 2004).

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52 53 453 **Conclusion**

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55 454 Water stress affected the seed yield dramatically under terminal drought conditions,
56 455 especially in sensitive genotypes (H77/833-2 and 841B). This was related to their yield

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3 456 architecture causing a decline in the number of productive panicles thereby effecting the seed
4 457 number, ultimately the total seed weight. The seed number started showing the effect of water
5 458 stress at levels of soil moisture where the 100-seed weight was still not affected and the
6 459 magnitude of the decrease in seed number was higher than the magnitude of the decrease in
7 460 the 100-seed weight. By contrast, the 100-seed weight was more affected in tolerant
8 461 genotypes by water stress than in sensitive genotypes, even though all the genotypes had very
9 462 severe 100-seed weight reductions at the most severe stress. Thus, retention of seed number
10 463 and sustained seed filling under water stress resulted in higher yield in tolerant parents
11 464 PRLT2/89-33 and 863B. However, these criteria may not be suited for unpredicted and pre-
12 465 flowering water stress conditions. IL-QTL's followed the pattern of tolerant parent
13 466 PRLT2/89-33 under water stress in their seed number and seed filling, which suggests that
14 467 the terminal drought tolerance QTL may have some role to play in the maintenance of a
15 468 higher number of seeds under water stress and a relatively higher grain size than recurrent
16 469 parent H77/833-2.
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Figure legends

Figure 1. Total seed weight of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar (\pm SE) indicates significant genotypic differences within the treatment ($P < 0.1$). The total seed weight was also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046, and ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS) conditions (i.e. 0.3 FTSW). Values are means (\pm SE) of each genotype harvested at 10, 20, and 30 DAF. Values were compared across harvest time for each genotype and harvest time values for with same letters above the bar were not significantly different ($P < 0.1$).

Figure 2. Panicle number of two pairs of pearl millet test cross hybrids: PRLT 2/89-33, and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar (\pm SE) indicates significant genotypic differences within treatment ($P < 0.05$).

Figure 3. Seed number of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on the x-axis were not significantly different. Star represented above the bar (\pm SE) indicates significant genotypic differences within the treatment ($P < 0.01$). Seed number was also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046, ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS) conditions (0.3 FTSW). Values are means (\pm SE) of each genotype harvested at 10, 20, and 30 DAF. Values were compared across harvest time for each genotype ($P < 0.05$) and harvest time values with same letters above the bar were not significantly different

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604 Figure 4. 100 seed weight of two pairs of pearl millet test cross hybrids PRLT 2/89-33 and
605 H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a well-watered
606 control (WW) and four water stress treatments imposed by maintaining soil moisture at 0.5,
607 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with same letters on
608 the x-axis were not significantly different. Star represented above the bar (\pm SE) indicates
609 significant genotypic differences within the treatment ($P < 0.05$). The 100 seed weight was
610 also monitored at different days after flowering (DAF, c and d) in two pearl millet test cross
611 hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and their NILs ICMR 01046,
612 ICMR 01029 (c) and in 863B and 841B (d) under well watered (WW) and water stress (WS)
613 conditions (0.3 FTSW). Values are means (\pm SE) of each genotype harvested at 10, 20, and 30
614 DAF. Values were compared across harvest time for each genotype ($P < 0.05$) and harvest
615 time values with same letters above the bar were not significantly different.

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617 Figure 5: Stover biomass (Stem + leaves) of two pairs of pearl millet test cross hybrids PRLT
618 2/89-33 and H77/833-2 (a), 863B and 841B (b) exposed to different water regimes, i.e. a
619 well-watered control (WW) and four water stress treatments imposed by maintaining soil
620 moisture at 0.5, 0.4, 0.3 and 0.2 FTSW (fraction of transpirable soil water). Treatments with
621 same letters on the x-axis were not significantly different. Star represented above the bar
622 (\pm SE) indicates significant genotypic differences within treatment ($P < 0.05$). The stover
623 biomass (leaf and stem) was also monitored at different days after flowering (DAF, c and d)
624 in two pearl millet test cross hybrids of PRLT2/89-33 (tolerant), H77/833-2 (sensitive) and
625 their NILs ICMR 01046, ICMR 01029 (c) and in 863B and 841B (d) under well watered
626 (WW) and water stress (WS) conditions i.e. 0.3 FTSW (fraction of transpirable soil water).
627 Values are means (\pm SE) of each genotype harvested at 10, 20, and 30 DAF. Values were
628 compared across harvest time for each genotype ($P < 0.05$) and harvest time values with same
629 letters above the bar were not significantly different.

630

631 Figure 6: Daily transpiration (Exp.1, March 2009) of two pairs of pearl millet test cross
632 hybrids PRLT 2/89-33 and H77/833-2 (a), 863B and 841B (c) exposed to different water
633 regimes, i.e. a well-watered control (WW) and four water stress treatments imposed by
634 maintaining soil moisture at 0.5, 0.4, 0.3, 0.2FTSW after flowering. NTR (normalized
635 transpiration ratio) of these four parental lines PRLT 2/89-33 and H77/833-2 (b), 863B and
636 841B (d) at 0.5 and 0.3 FTSW (fraction of transpirable soil moisture).

637

638 Fig. 7: Total carbohydrates from the penultimate internodes of two pearl millet test cross
639 hybrids of parental lines PRLT2/89-33 (tolerant), H77/833-2 (sensitive), their NILs ICMR
640 01046, ICMR 01029 (Exp.2, April 2010) and 863B (tolerant), 841B(sensitive) parental
641 lines harvested at maturity from well watered (WW) and water stress (WS) i.e. 0.3 FTSW
642 (fraction of transpirable soil water).

643

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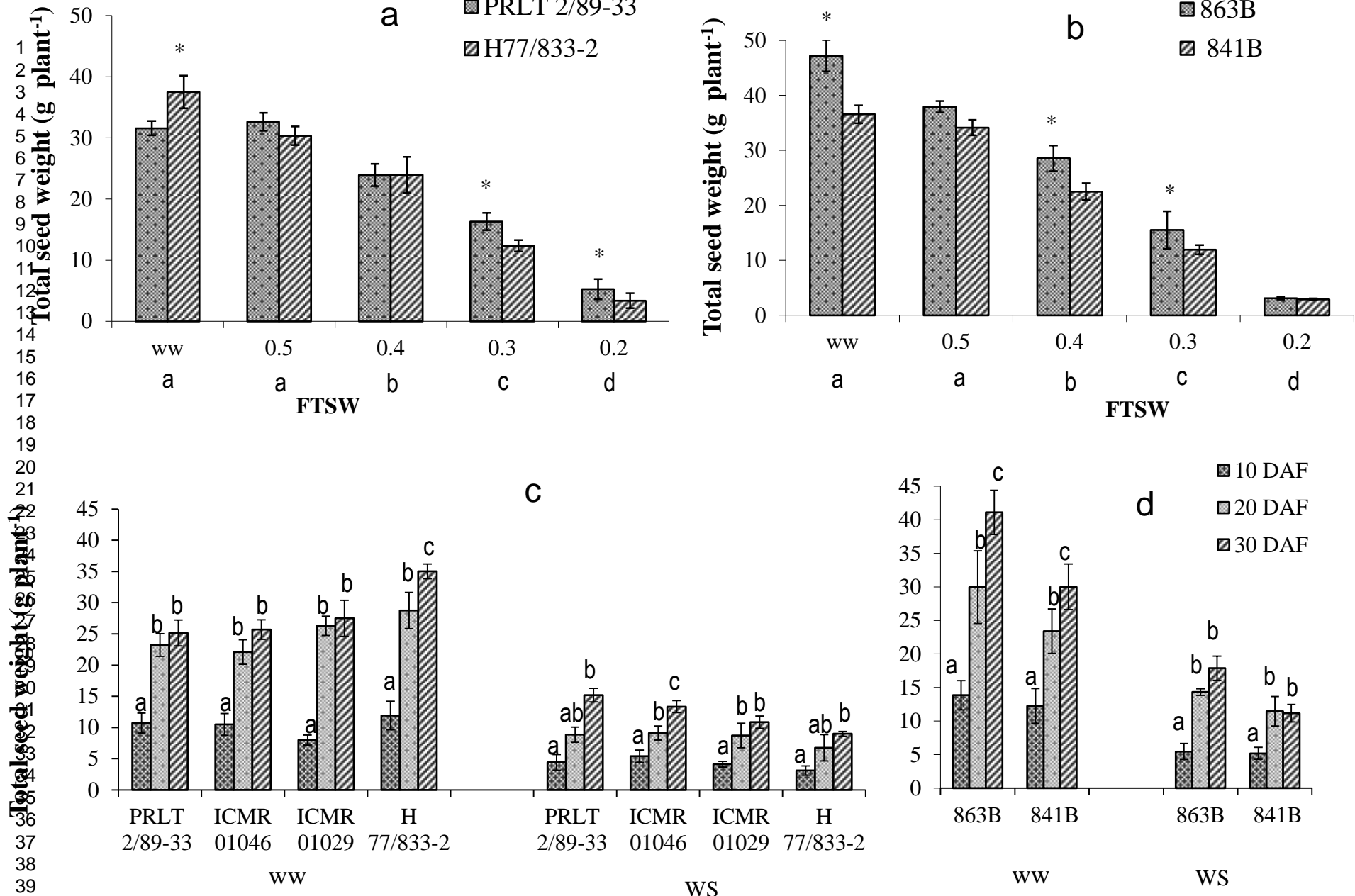


Figure 1

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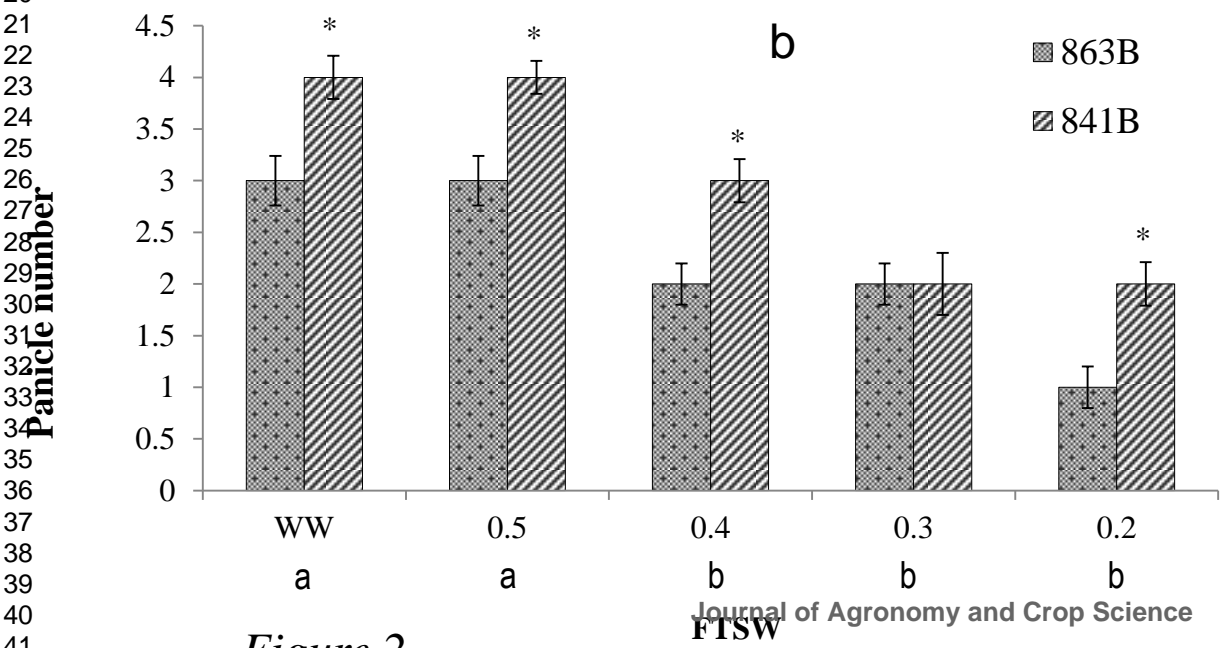
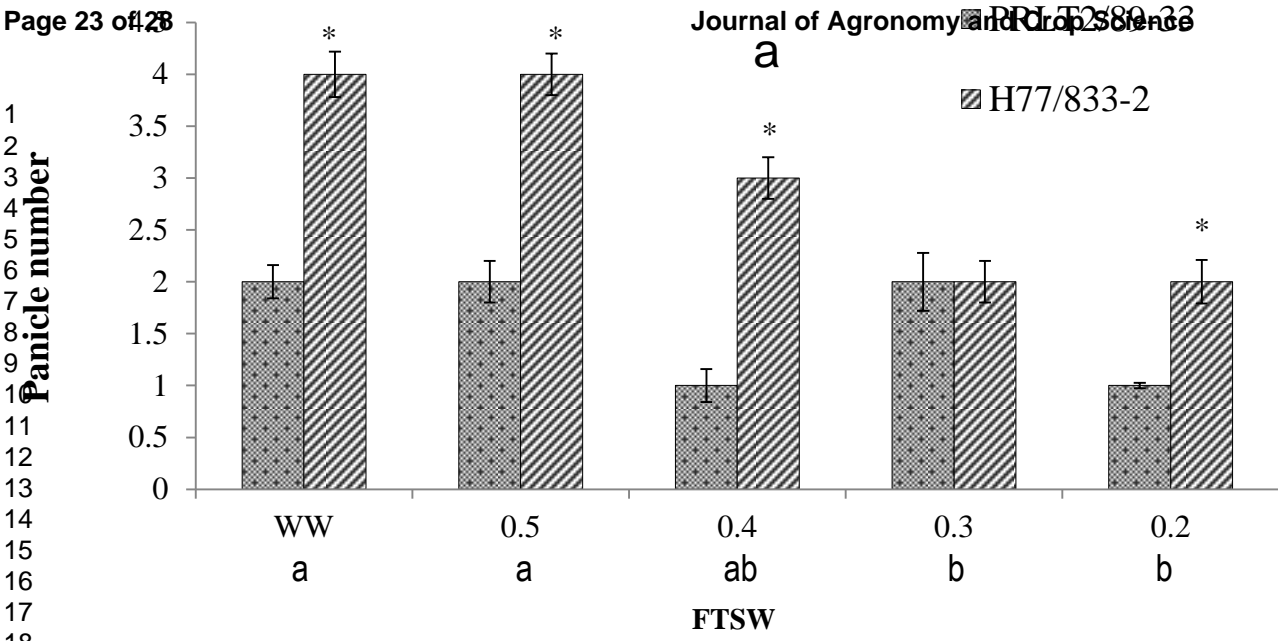


Figure 2

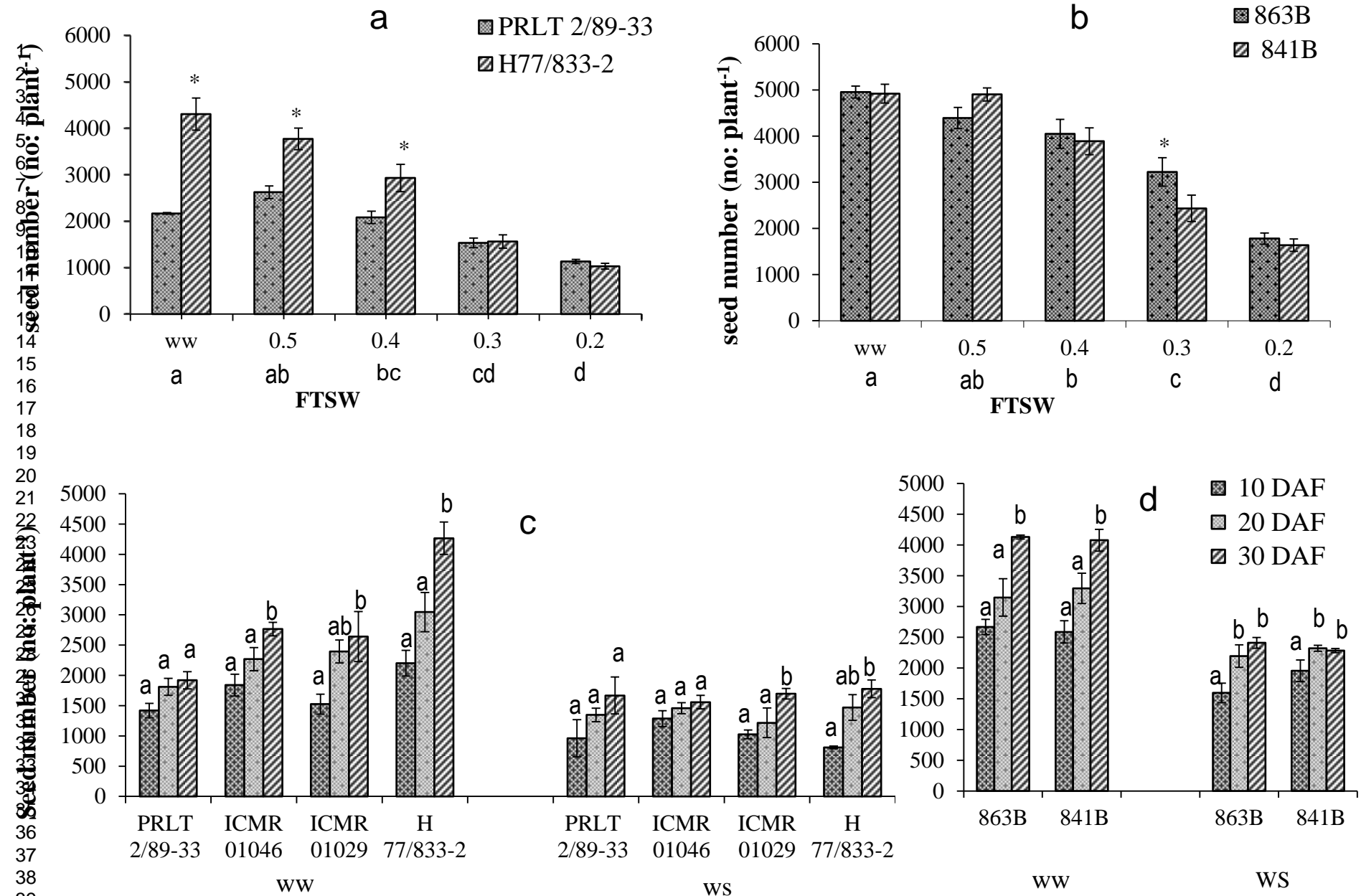
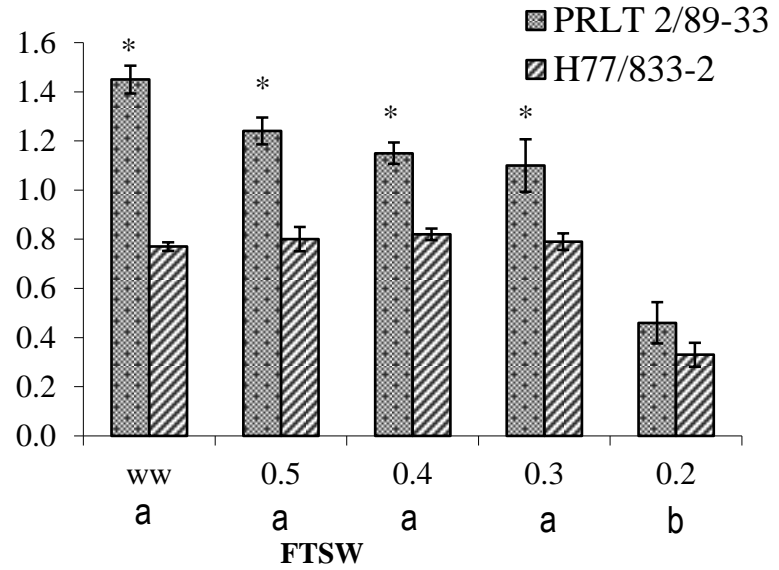
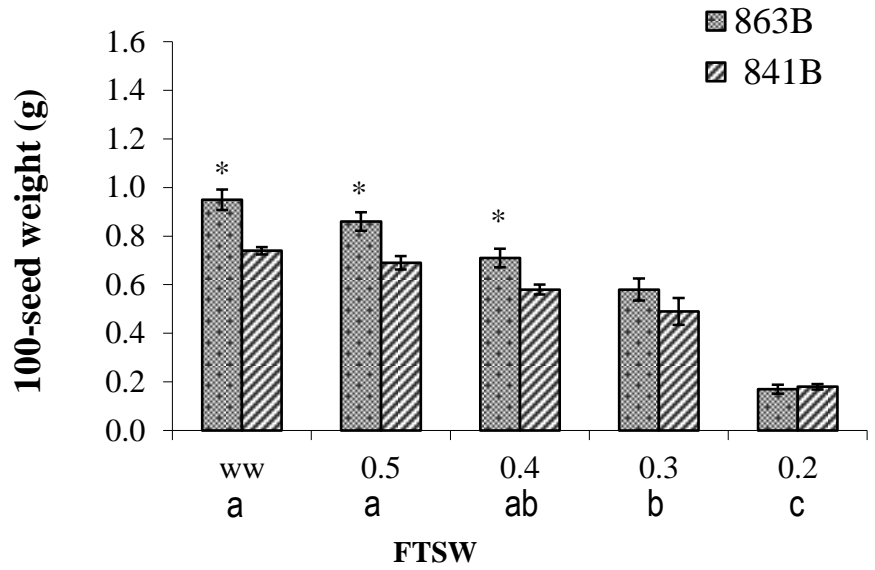


Figure 3

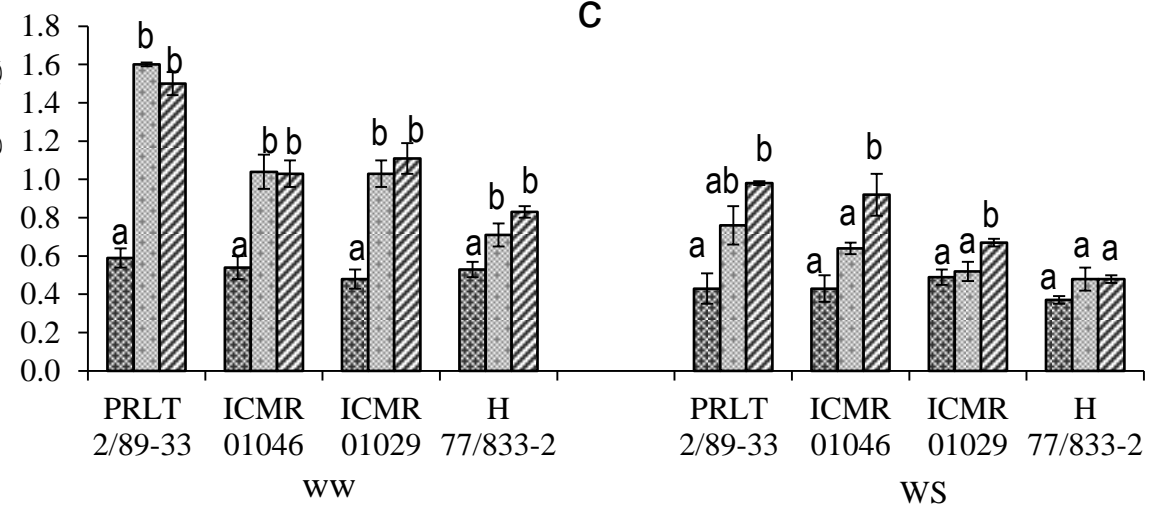
a



b



c



d

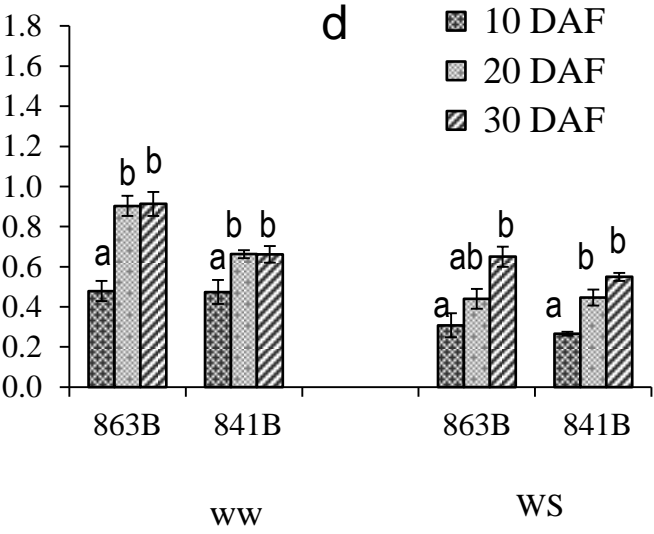


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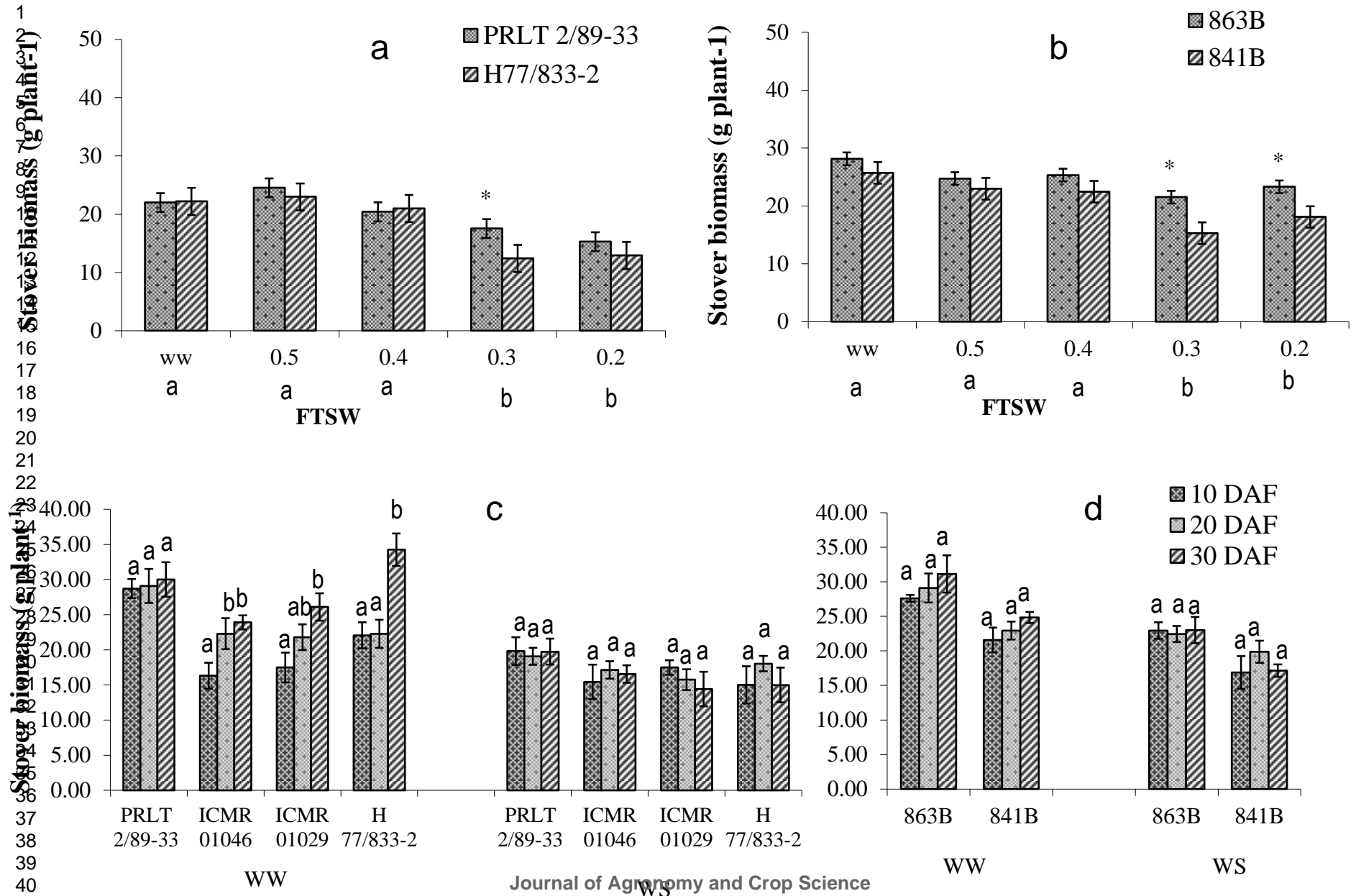


Figure 5

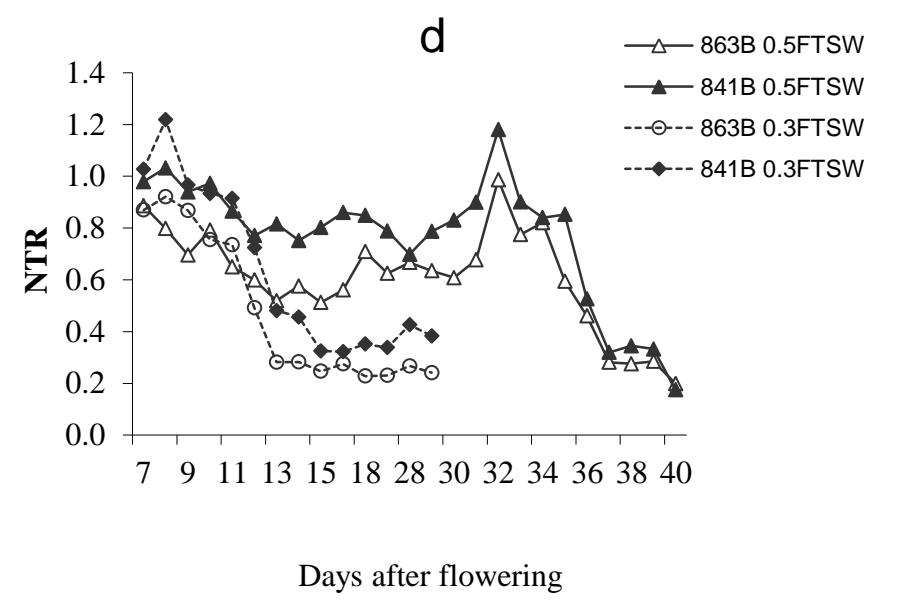
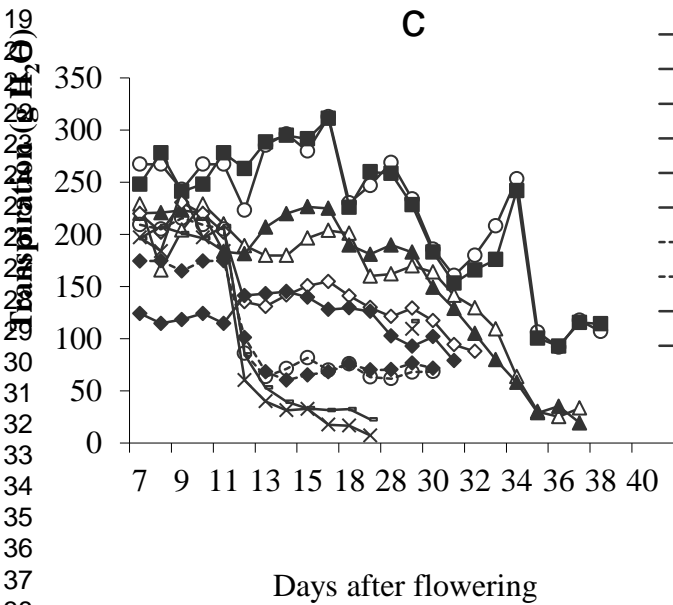
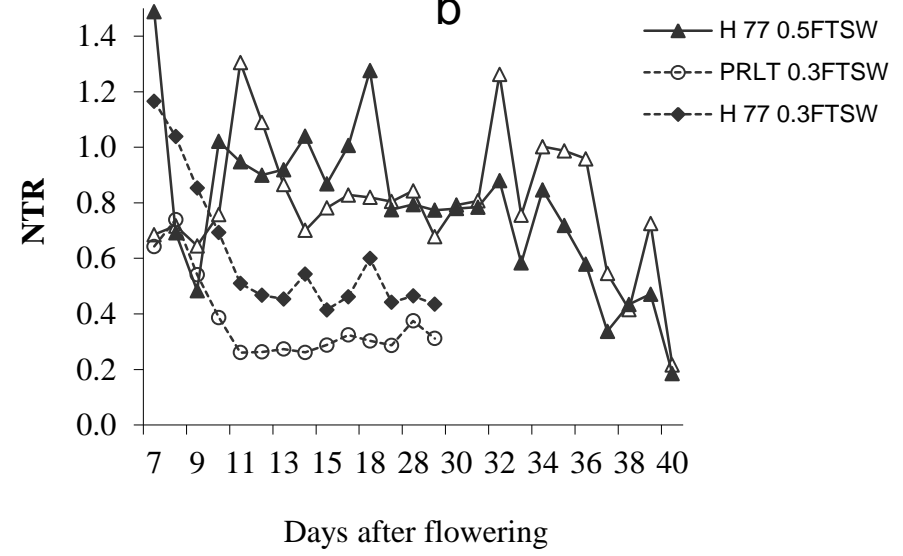
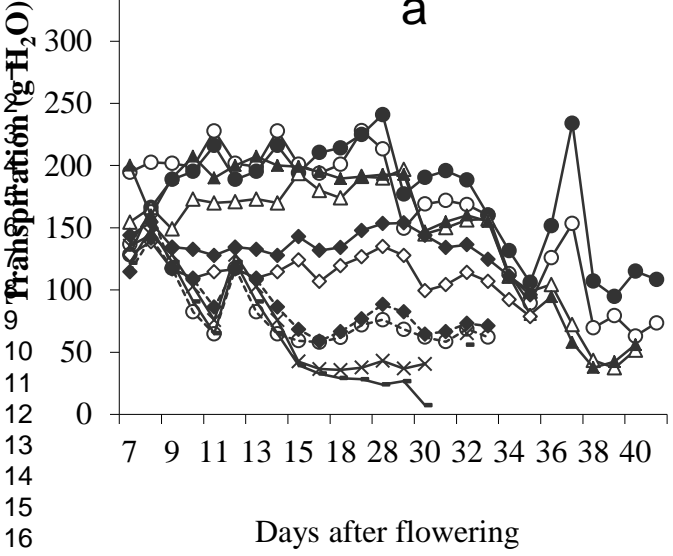


Figure 6

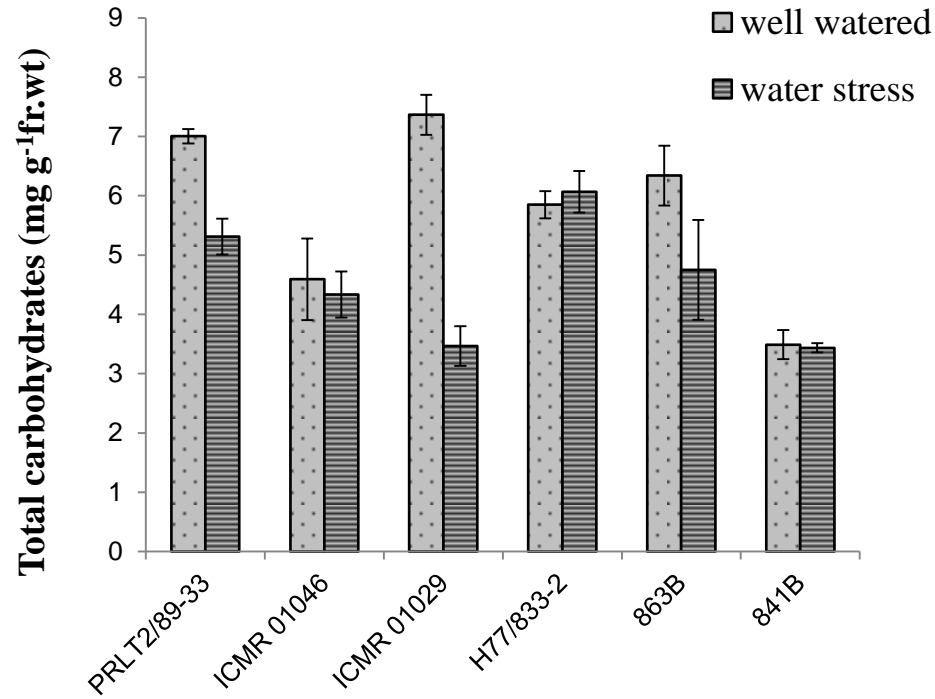


Figure 7