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Validation of quantitative trait loci for aluminum tolerance in Chinese wheat landrace FSW

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1 Validation of quantitative trait loci for aluminum tolerance in Chinese wheat landrace

2 FSW

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18

19 **Abstract** Aluminum (Al) toxicity is one of the major constraints for wheat production
20 in acidic soils worldwide and use of Al-tolerant cultivars is one of the most effective
21 approaches to reduce Al damage in the acidic soils. A Chinese landrace, FSW, shows a
22 high level of tolerance to Al toxicity and a mapping population of recombinant inbred
23 lines (RILs) was developed from a cross between FSW and Al-sensitive US spring
24 wheat cultivar Wheaton to validate the quantitative trait loci (QTL) previously
25 identified in FSW. The mapping population was evaluated for net root growth (NRG)
26 during Al stress in a nutrient solution culture and hematoxylin staining score (HSS) of
27 root tips after Al stress. After 132 simple sequence repeat (SSR) markers from three
28 chromosomes that were previously reported to have the QTLs were analyzed in the
29 population, two QTLs for Al tolerance from FSW were confirmed. The major QTL on
30 chromosome 4DL co-segregated with the Al-activated malate transporter gene
31 (*ALMT1*), however, sequence analysis of the promoter region (Ups4) of *ALMT1* gene
32 indicated that FSW contained a marker allele that is different from the one that was
33 reported to condition Al tolerance in the Brazilian source. Another QTL on
34 chromosome 3BL showed a minor effect on Al tolerance in the population. The two
35 QTLs accounted for about 74.9% of the phenotypic variation for HSS and 72.1% for
36 NRG and demonstrated an epistatic effect for both HSS and NRG. SSR markers
37 closely linked to the QTLs have potential to be used for marker-assisted selection
38 (MAS) to improve Al tolerance in wheat breeding programs.

39 **Keywords** Chinese landrace • aluminum tolerance • simple sequence
40 repeats • QTL mapping

42 **Introduction**

43 Aluminum (Al) toxicity is a major limiting factor for crop production in acidic soils
44 worldwide. When soil pH is lower than 5, exchangeable Al³⁺ is released into the soil
45 solution, inhibiting normal root growth and function (Samac and Tesfaye 2003) and,
46 therefore causes a significant reduction in crop yield. Over 40% of the world's
47 potentially arable lands are acidic (von Uexküll and Mutert 1995; Bot et al. 2000)
48 with up to 60% of them in developing countries (Kochian et al. 2005). Due to
49 extensive crop production, the area of acidic soils is quickly increasing (Guo et al.
50 2010). Although irrigation or application of lime to acidic soils can increase soil pH to
51 relieve Al toxicity, the high cost associated with transportation of lime to destination
52 limits widespread adoption of this practice. Fortunately, significant genetic variation
53 in Al tolerance has been reported in wheat (Stodart et al. 2007; Zhou et al. 2007a; Hu
54 et al. 2008), and growing Al-tolerant cultivars is the most cost-effective approach to
55 improve wheat production in acidic soils.

56 Inheritance of Al tolerance in wheat has been extensively studied especially from
57 Brazilian source such as BH1146 and Atlas 66 (Kochian et al. 2005; Samac and
58 Tesfaye 2003; Tang et al. 2002, Ma et al. 2005). However, results on number and
59 locations of genes/QTLs for Al tolerance in wheat are still equivocal. Several studies
60 indicated that Al tolerance in wheat was under monogenic control (Raman et al. 2005;
61 Riede and Anderson 1996), whereas others suggested that multiple genes might be
62 involved in enhancing Al tolerance in some wheat genotypes (Berzonsky 1992; Cai et
63 al 2008; Zhou et al. 2007b). Also, the Al tolerance in Asian accessions might not be

64 the same as that from Brazilian sources (Hu et al. 2008; Raman et al. 2008; Zhou et al.
65 2007a). For example, a Chinese wheat landrace, FSW, showed a similar level of Al
66 tolerance to Atlas 66 as measured by hematoxylin staining, but it had a different
67 haplotype pattern for the markers derived from *ALMT1* (Hu et al. 2008), a gene
68 encoding an Al-activated malate transporter cloned from the Brazilian source (Sasaki
69 et al. 2004). In addition, different genetic backgrounds may affect expression of
70 tolerance genes that are from the same source. In Atlas 66, a QTL on chromosome
71 4DL was mapped in both populations of Atlas 66/Century and Atlas 66/Chisholm, but
72 a minor QTL on chromosome 3BL was detected only in Atlas 66/Chisholm (Ma et al.
73 2005; Zhou et al. 2007b).

74 Malate release from root tips has been considered as the major mechanism of Al
75 tolerance in wheat (Sasaki et al. 2004). The major QTL on 4DL cosegregated with
76 *ALMT1* in several populations (Ma et al. 2005; Raman et al. 2005; Sasaki et al. 2004).
77 Several markers (*ALMT1*-CAP, SSR3a, and SSR3b) were developed from the
78 gene-coding region for marker-assisted selection (MAS) of the 4DL QTL (Raman et
79 al. 2006). However, these markers were only effective in some crosses but not others
80 (Zhou et al. 2007b). A new marker has been developed from the promoter region of
81 *ALMT1* and reported as a diagnostic marker for Al tolerance on 4DL (Sasaki et al.
82 2006; Raman et al. 2008). In FSW, QTLs were initially mapped on 4DL, 3BL and 2A
83 in a population from a cross between FSW and a Chinese line ND35 (Cai et al. 2008).
84 However, these QTLs have not been validated in other populations. The objectives of
85 this study were to validate, in FSW, the effect of Al tolerance QTL that have been

86 previously identified in other sources, to investigate haplotype patterns of *ALMT1*
87 marker alleles and to develop high-throughput PCR-based markers for MAS of Al
88 tolerance in wheat breeding programs.

89

90 **Materials and methods**

91

92 Plant materials and evaluation of Al tolerance

93 A mapping population of 217 F₆ recombinant inbred lines (RILs) was derived from
94 the cross FSW / Wheaton by the single-seed-descent method. FSW is an Al-tolerant
95 landrace from China, and Wheaton is an Al-sensitive cultivar from Minnesota, U.S.A.

96 To evaluate Al tolerance of the RILs, wheat seeds were germinated on wet filter
97 papers in petri dishes at 4° C for 72 h. Three germinating seeds with similar
98 appearance were transferred onto a nylon wire net on open bottom of a plastic cup. A
99 plastic cup holder was used to support the cups floating on deionized water at room
100 temperature (20-23° C) with a 16 h photoperiod using fluorescent lights. Two bubble
101 rods in the water connected to an air pump provided aeration during the culture period.
102 After 48 h of hydroponic culture, the deionized water was replaced with nutrient
103 solution (pH 4.0) consisting of 4 mM CaCl₂, 6.5 mM KNO₃, 2.5 mM MgCl₂.6H₂O,
104 0.4 mM NH₄NO₃, 0.1 mM (NH₄)₂SO₄, and 0.36 mM AlK(SO₄)₂.12 H₂O. Reactions of
105 parents and RILs to Al stress were evaluated by measuring root growth during Al
106 stress and degree of hematoxylin staining of Al-treated root tips. The principal root of
107 each seedling was measured twice after two days of hydroponic culture and

108 three-days of Al treatment in nutrient solution to calculate root length difference
109 between the two measurements as net root growth (NRG). Root hematoxylin stain
110 measures the Al amount that entered into plant roots during Al treatment and has been
111 widely used to measure plant Al tolerance (Ma et al, 2005; Polle et al. 1978). After the
112 second measurement of root length, excess Al^{3+} on the root surface was rinsed off in
113 de-ionized water for 1 h, with three replacements. Clean roots were then submerged in
114 a hematoxylin solution containing 0.2% hematoxylin (w/v) and 0.02% (w/v) $NaIO_3$
115 for 15 min, followed by rinsing the roots with de-ionized water three to four times.
116 The stained root tips of each stained seedling were visually scored as hematoxylin
117 stain score (HSS) using a 1-3 grading scale: no stain on root tips as 1, lightly stained
118 as 2, and heavily stained as 3 (Ma et al. 2005). The experiments were repeated twice
119 with three and four replicates (cups), respectively, using a randomized complete block
120 design. In each experiment, an additional replication was used as control in which the
121 culture solution did not contain any Al^{3+} .

122 After hematoxylin staining, wheat seedlings were grown in a greenhouse for one
123 week to harvest leaf tissue of each seedling for DNA isolation. Leaf tissue was
124 collected in a 1.5-mL tube and dried in a freeze drier (Thermo Fisher, Waltham, MA,
125 USA) for 2 d. Tubes containing dried tissue were shaken at 25 times/s for 4 min in a
126 Mixer Mill (Retsch GmbH, Haan, Germany) with a 3.2 mm stainless steelbead in each
127 tube.

128

129 Marker analysis

130 Genomic DNA was extracted using the Cetyltrimethyl ammonium Bromide (CTAB)
131 method (Saghai-Marooif et al. 1984). A total of 132 pairs of SSR primers from the
132 chromosomes that were previously reported to have QTLs for Al tolerance were
133 selected to screen parents (Cai et al. 2008; Zhou et al. 2007b; Ma et al. 2005) and
134 polymorphic primers were further analyzed in the F₆ RIL population.

135 For SSR analysis, a 10- μ L PCR mixture contained 40 ng of template DNA, 2.5 mM
136 MgCl₂, 200 μ M each of dNTPs, 50 nM of forward tailed primer, 100 nM of reverse
137 primer and 50 nM of M13 fluorescent-dye labeled primer, 1 \times PCR buffer, 1 U of *Taq*
138 polymerase. A touchdown PCR program was used for PCR amplification, in which
139 the reaction mixture was incubated at 95 °C for 5 min, then continued for 5 cycles of 1
140 min of denaturing at 96 °C, 5 min of annealing at 68 °C with a decrease of 2 °C in each
141 subsequent cycle, and 1 min of extension at 72 °C . For another 5 cycles, the annealing
142 temperature started at 58 °C for 2 min with a decrease of 2 °C for each subsequent
143 cycle. Then, reactions went through an additional 25 cycles of 1 min at 96 °C, 1 min at
144 50 °C, and 1 min at 72 °C with a final extension at 72 °C for 5 min. PCR products were
145 analyzed in an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City,
146 CA, USA).

147

148 Data analysis

149 Marker data collected from the ABI DNA Analyzer were further processed using
150 GeneMarker version 1.5 (SoftGenetics LLC, State College, PA, USA) and rechecked
151 twice manually for accuracy. Genetic linkage groups of SSR markers were

152 constructed using JoinMap3.0 (Van Ooijen and Voorrips 2001). Recombination
153 frequencies were converted into centiMorgans (cM) using the Kosambi function
154 (Kosambi 1944). The threshold value of logarithm of odd (LOD) score was set at 3.0
155 to claim linkage between markers with a maximum fraction of recombination at 0.4.
156 WinQTLCart2.5 (Wang et al. 2007) was used for QTL mapping. Genome-wide LOD
157 threshold values for declaring a significant QTL at $P < 0.05$ were obtained by running
158 1,000 times of permutations separately for NRG and HSS traits (Doerge and Churchill
159 1996). Analysis of variance, heritability of Al tolerance traits and determination
160 coefficient (R^2) were calculated using SAS system Version 9.1 (SAS Institute, Inc.,
161 2003, Cary, NC, USA).

162

163 **Results**

164

165 Variation in root responses of RILs and their parents to Al stress

166 The roots of FSW were longer (3.3 cm) than those of Wheaton (0.6 cm) after 72 h of
167 hydroponic culture in a nutrient solution containing 0.36 mM Al^{3+} . After 3 d of Al
168 treatment, the root tips of Wheaton were fully stained by hematoxylin (grade 3),
169 whereas those of FSW were not stained (grade 1). In non-Al controls, Wheaton and
170 FSW showed similar root lengths and hematoxylin stain scores. Therefore, the Al
171 concentration used in this study was appropriate for differentiating the tolerant
172 genotypes from the sensitive genotypes by measuring either NRG or HSS.

173 The frequency distribution of NRG of the RILs under Al stress was continuous

174 with the major peak toward Wheaton (Fig. 1). A similar distribution was observed for
175 HSS (Fig. 2). A highly significant correlation coefficient ($r=0.87$, $P<0.01$) was
176 observed between NRG and HSS in the mapping population. The correlations
177 between untreated root length and NRG, HSS were low ($r=0.21$ and 0.20 , respectively)
178 and not significant in the RIL population. Therefore, NRG and HSS were independent
179 of variation in root growth under non-AI-stressed conditions among RILs. Variance
180 analysis showed that the effects of RILs were significant in both NRG and HSS
181 (Table 1). Heritability was high for both NRG (0.88) and HSS (0.87), and thus, only a
182 few genes may be involved in AI tolerance in the population.

183

184 QTL for AI tolerance in FSW

185 After 132 SSR primers were screened, 35 were polymorphic between parents and
186 further analyzed in the F_6 RIL population. A total of 24 markers were mapped in the 3
187 linkage groups spanning 138.7 cM of genetic distance. The first group had 9 SSRs
188 spanning 41.8 cM on chromosome 3BL, the second had 12 SSRs spanning 88.2 cM
189 on chromosome 4DL, and the third had only 3 SSRs spanning 8.7 cM on chromosome
190 2A. These three linkage maps were used for further QTL analysis. Interval mapping
191 identified two QTLs for AI tolerance on chromosomes 4DL and 3BL. The QTL on
192 4DL showed a major effect on both NRG and HSS, whereas the QTL on 3BL had a
193 minor effect on NRG and HSS (Fig. 3). The QTL on 4DL co-segregating with
194 *Xwmc331* was flanked by the markers *Xups4* and *Xgdm125*, with R^2 values of 65.7%
195 for NRG and 70.1% for HSS and LOD values 57.8 for NRG and 64.9 for HSS. The

196 QTL on 3BL was flanked by the markers *Xbarc344* and *Xbarc164*, with R^2 values of
197 3.7% for NRG and 2.7% for HSS, with LOD value 7.8 for NRG and 6.7 for HSS
198 (Table 2).

199 To analyze the effect of the two QTLs on AI tolerance, the closest markers
200 *Xwmc331* on 4DL and *Xbarc344* on 3BL were selected to represent the two QTLs
201 (Fig. 4). Four possible combinations of the two QTLs are: 4DL+/3BL+, 4DL+/3BL-,
202 4DL-/3BL+, 4DL-/3BL-, in which 4DL+ and 3BL+ represent AI tolerance marker
203 alleles of QTLs from 4DL and 3BL of FSW, respectively, and 4DL- and 3BL-
204 represent corresponding AI-sensitive marker alleles from Wheaton. Mean
205 comparisons of these genotype combinations indicated that combination of these two
206 QTLs increased NRG by 2.6 cm and decreased HSS by 1.8 relative to the genotype
207 with AI-sensitive haplotype of the marker alleles on both 4DL and 3BL. In the
208 presence of the AI-tolerance marker allele on 4DL, the 3BL marker allele associated
209 with AI tolerance increased NRG by 1 cm, whereas it only increased about 0.2 cm
210 without the 4DL marker allele linked to AI tolerance. Similarly, the tolerance allele on
211 3BL decreased by 0.6 in HSS in the presence of 4DL allele and very little when
212 marker allele associated with AI tolerance on 4DL was absent (Fig. 4). These two
213 QTLs for AI tolerance appeared to have epistatic effect on NRG and HSS.

214 Two *ALMT1* gene markers, *Xups4* and *Xssr3a*, were polymorphic between the two
215 parents and they were used to analyze the RILs. *Xups4* amplified two different sizes
216 of amplicons between AI-tolerant FSW and sensitive Wheaton. The size of 471 bp
217 allele was associated with AI-tolerant genotypes, whereas the 440 bp allele was

218 associated with AI-sensitive genotypes in the population. The correlation coefficient
219 of the *Xups4* allele with HSS and NRG were 84% and 83%, respectively, in the RIL
220 population. *Xssr3a* amplified a 225 bp fragment in FSW and a 223 bp fragment in
221 Wheaton. The correlation coefficient of the *Xssr3a* allele with HSS and NRG are 83%
222 and 82%, respectively, in the RIL population. *Xssr3b* did not amplify any alleles in
223 two parents and the RILs, and thus it was not analyzed further.

224

225

226 **Discussion**

227

228 Evaluation of Al tolerance

229 Al tolerance of wheat is usually evaluated in acidic soils under field conditions.

230 Inconsistent phytotoxicity and pH value among the plots may induce significant

231 environmental variations (Ma et al. 2005). Thus field tests may not provide consistent

232 results for the proper comparison. An alternative method for evaluating Al tolerance

233 using hydroponic culture provides a strict control in nutrient solution containing a

234 toxic level of Al and pH, and can provide non-destructive measurements in large

235 populations. Therefore, it has been widely used in genetic studies (Polle et al. 1978;

236 Ma et al. 2006; Guo et al. 2007; S. Navakode et al. 2009). With this method, net root

237 growth of Al-stressed seedling has been measured to reflect plant tolerance to Al

238 toxicity in several studies (Parker and Pedler 1998; Zhou et al. 2007a). Hematoxylin

239 staining can measure the extent of Al accumulation in root cells and has been widely

240 used to evaluate Al tolerance in several crops (Delhaize et al. 1993; Cancado 1999;

241 Anas 2000). In this study, both NRG and HSS were used to measure Al tolerance of

242 parents and the RIL mapping population. A high correlation between the two traits

243 was observed ($r=0.87$, $P<0.01$).

244 The two parents showed a large contrast in NRG and HSS. Significant variations

245 in NRG and HSS were observed among the RILs with high heritability of both

246 measurements. QTL for HSS and NRG were mapped on the same chromosome

247 positions. The two QTLs on 4DL and 3BL together accounted for about 74.9 % of the

248 phenotypic variation for HSS and 72.1% for NRG. Results suggested that both HSS
249 and NRG were reliable measurements for the mapping study of Al tolerance.

250

251 Inheritance of Al tolerance in wheat

252 Wheat is the best-characterized species and genetic system for analyzing Al tolerance
253 (Kochian et al. 2004). Several studies that used the Brazilian sources of tolerance such
254 as BH1146 and Atlas 66 postulated that Al tolerance segregated as a single dominant
255 locus. Riede and Anderson (1996) first mapped the gene as Alt_{BH} on 4DL of BH1146
256 using restricted fragment length polymorphism and concluded that this gene was fully
257 responsible for Al tolerance in BH1146. Ma et al. (2005) identified a QTL for Al
258 tolerance on the same chromosome region of Atlas 66 using a RIL population from
259 Atlas 66/Century. However, several other studies suggested that at least two loci
260 might be involved in Al tolerance in Atlas 66 (Garvin and Carver 2003; Tang et al.
261 2002; Zhou et al 2007b). Several studies using wheat genetic stocks including
262 deletion lines, nullitetrasonics, and ditelosomics also supported multigenic controls
263 of Al tolerance (Aniol and Gustafson 1984; Aniol 1990; Ma et al. 2006; Papernik et al.
264 2001). In this study, two QTLs on 4DL and 3BL were identified, which agrees with
265 Zhou et al. (2007b). In that study, the minor QTL on 3BL of Atlas 66 accounted for
266 11.1% of the phenotypic variation for HSS and 8.6% for NRG. Cai et al. (2008) used a
267 population developed by crossing FSW to a Chinese dwarf line ND35 and reported
268 that the QTL on 3BL showed a major effect on Al tolerance with $R^2 = 47.0\%$ for HSS
269 and 41.7% for NRG. However, the QTL showed a much smaller effect on Al tolerance

270 in the Wheaton background ($R^2=2.7\%$ and 3.7% for HSS and NRG, respectively) in
271 this study although the QTL on 3BL in this study was mapped on the same
272 chromosome region as that reported by Cai et al. (2008). This 3BL QTL was detected
273 in different sources of Al-tolerant germplasm and same source in different genetic
274 backgrounds, and therefore is more likely a 'real' QTL. However this QTL appears to
275 be less stable than the one on 4DL. The minor QTL for HSS and NRG on
276 chromosome 2A reported by Cai et al. (2008) was not detected in this study although
277 the markers linked to the QTL reported by Cai et al. (2008) were polymorphic in the
278 current population. It is also possible that other minor genes may be involved in Al
279 tolerance in the population because only three previously reported chromosome
280 regions were screened in this study.

281

282 Marker allele for *ALMT1* in FSW

283 *ALMT1* on 4DL has been considered a major contributor to Al tolerance in several
284 germplasm lines (Raman et al. 2005; Ma et al. 2005; Sasaki et al. 2004; Zhou et al.
285 2007b) and it has been used as a major Al tolerance gene in MAS in breeding
286 programs where Al tolerance is a major breeding objective. Raman et al. (2005)
287 studied the structure and chromosomal location of *ALMT1* and concluded that Al
288 tolerance in a diverse range of wheat genotypes is primarily conditioned by *ALMT1*.

289 In this study, the QTL with the largest effect on Al tolerance in FSW was also
290 mapped to a similar location as that in Atlas 66 (Ma et al. 2005; Zhou et al. 2007b).

291 *ALMT1* as represented by *Xups4* was also mapped in the QTL region in FSW that

292 confirmed the previous report (Cai et al. 2008). Interestingly, *Xwmc331* was the
293 closest marker for the QTL, not *Xups4*, and *Xgdm125* and *Xups4* flanked the QTL for
294 both traits, which agrees with Cai et al. (2008) who mapped the major QTL between
295 *Xgdm125* and *Xups4* in FSW/ND35 population. *Xups4* is a sequence upstream from
296 *ALMT1* in wheat.

297 Sasaki et al. (2006) further investigated the promoter structure of *ALMT1* and
298 concluded that expression of Al tolerance is mainly conditioned by the variation in
299 promoter size. The germplasm that amplified large fragments (706 to 1229 bp) by
300 *Xups4* from the promoter region of *ALMT1* were considered Al tolerant whereas the
301 germplasm that amplified 469 bp or smaller fragments were considered to be sensitive
302 to Al stress. In this study, FSW amplified a 471 bp amplicon, a sensitive allele based
303 on Sasaki et al. (2006), but showed Al tolerance. However, Sasaki et al. (2006) also
304 noticed that Japanese lines showed a weak correlation between *ALMT1* expression
305 and Al tolerance. This suggested that the mechanisms of Al tolerance in FSW might
306 be different from that of the Brazilian source. FSW may have a different mechanism
307 in regulating expression of *ALMT1* or the *ALMT1* promoter may not be the key
308 molecular regulator for the *ALMT1* expression in FSW. It is also possible that some
309 other factors may be involved in the control of malate efflux in addition to the level of
310 *ALMT1* expression (Sasaki et al. 2006; Raman et al. 2005).

311 All three 4DL markers (*Xwmc331*, *Xups4* and *Xgdm125*) that were polymorphic in
312 FSW/Wheaton population were mapped in the QTL region showing a very large
313 effect on Al tolerance. *Xwmc331* and *Xgdm125* are SSR markers and suitable for

314 high-throughput analysis, and therefore, they can be used for MAS. *Xups4* is a gene
315 marker, and should be the best marker for MAS. AI tolerant FSW amplified a smaller
316 fragment of *Xups4* (453bp after removal of a 18bp M13 tail) that was considered the
317 allele associated with AI-sensitivity (Sasaki 2006) in Brazilian sources. Therefore the
318 amplicon size of *Xups4* cannot be used as the only selection criterion for the 4DL
319 QTL resistance allele. However, it still is an informative marker for the 4DL QTL if it
320 is polymorphic in a breeding population. Previously, a cleaved amplified
321 polymorphism (CAP) marker has been used as diagnostic marker for the 4DL QTL in
322 marker-assisted breeding for AI tolerance (Zhou et al. 2007b; Ma et al. 2005), but it
323 requires an additional step of restriction digestion after PCR amplification (Raman et
324 al. 2006). Thus, it can be replaced with *Xwmc331* or *Xups4* when FSW is used as an
325 AI-tolerant source.

326 In a summary, two QTLs for AI tolerance previously mapped in other populations
327 were confirmed in a new FSW population. The major QTL on chromosome 4DL
328 co-segregated with the AI-activated malate transporter gene (*ALMT1*), but it was a
329 different allele from the one previously reported to condition AI tolerance, was
330 identified in FSW. Another QTL on chromosome 3BL showed a minor effect on AI
331 tolerance in the population. The two QTLs accounted for about 74.9% of the
332 phenotypic variation for HSS and 72.1% for NRG. DNA markers closely linked to the
333 QTLs should be useful for MAS to improve AI tolerance in wheat breeding programs.

334

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347

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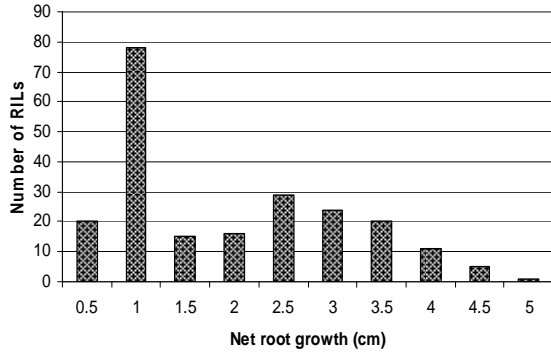
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449

450 **Figure 1**



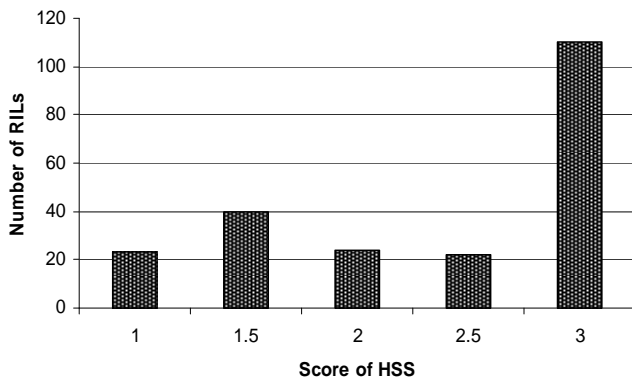
451

452 **Fig. 1** Frequency distribution of net root growth (NRG) for 217 F6 recombinant
453 inbred lines from the cross FSW/Wheaton after 72 h of AI stress. Arrows point to
454 mean NRG for parents FSW (right) and Wheaton (left).

455

456

457 **Figure 2**

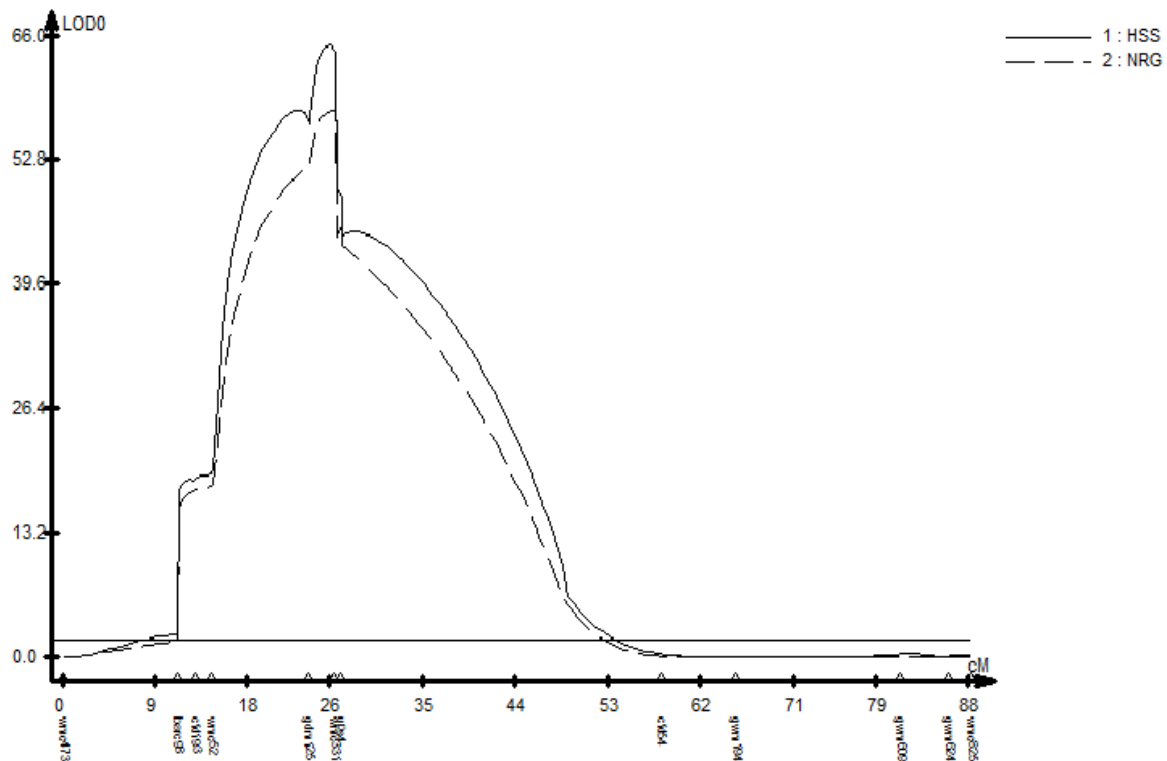


458

459 **Fig. 2** Frequency distribution of hematoxylin stain score (HSS) for RILs from the
460 cross FSW/Wheaton after 72 h of AI stress. Arrows point to mean HSS of HSS for
461 parents Wheaton (right) and FSW (left).

462

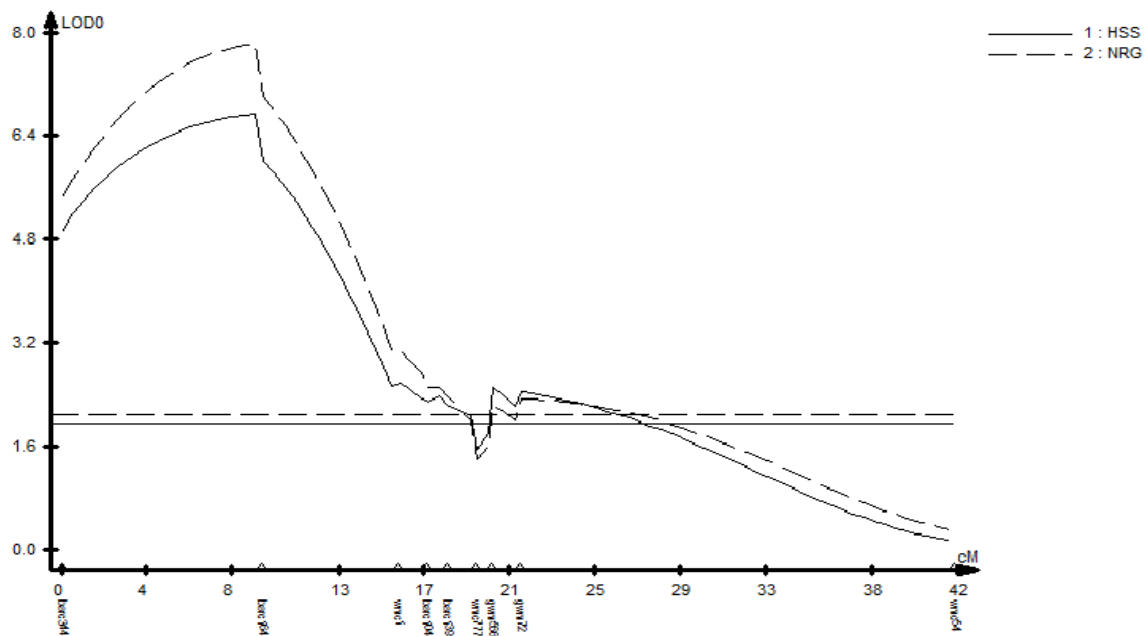
463 **Figure 3 A**



464

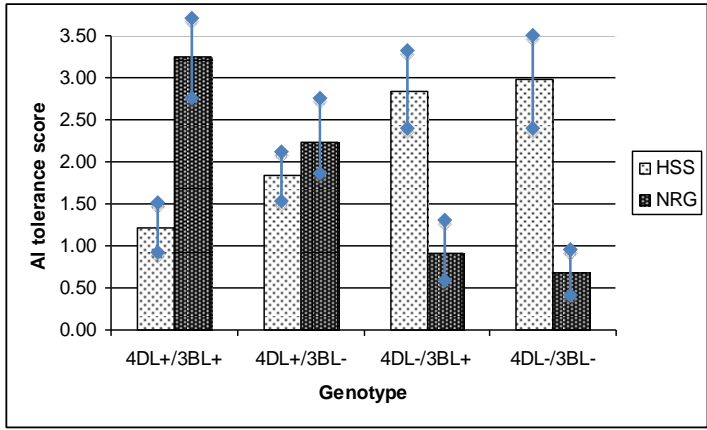
465 **Fig. 3 A.** Major QTL on 4DL and B. a minor QTL on 3BL.

466 **Figure 3 B**



467

468 **Figure 4**



469

470 **Fig. 4** Effect of 4DL and 3BL QTLs on AI tolerance in RIL population from the cross
471 FSW/Wheaton. 4DL+ and 3BL+ represent AI resistant marker alleles of the QTLs
472 from 4DL and 3BL of FSW respectively, and 4DL- and 3BL- represent AI sensitive
473 marker alleles of the two QTLs from Wheaton, respectively. NRG and HSS represent
474 net root growth (cm) and hematoxylin staining score, respectively. Lines are standard
475 deviations.

Table 1 Variance components and heritability for net root growth (NRG) and hematoxylin stain score (HSS) in the recombinant inbred population derived from the cross FSW/Wheaton

Source	DF	SS	MS	F-value	h^2
NRG					
Experiment	1	97.39		97.39	298.89**
RILs	216	1929.00	8.93	27.41**	0.88
Experiment*RILs	216	236.69	1.10	3.36**	
Error	1057	344.41	0.33		
Total	1490	2607.39			
HSS					
Experiment	1	2.87	2.87	17.49**	
RILs	216	892.45	4.13	25.21**	0.87
Experiment *RILs	216	120.26	0.56	3.40**	
Error	1059	173.56	0.16		
Total	1492	1189.14			

476

477