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Protocol for early vigour QTL mapping

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Material preparing

1 Parents, provided by CIMMYT:

durum wheat DOY1 x *Ae. tauschii* AT333, doubled haploids Syn79 by colchicine application

durum wheat DOY1 x *Ae. tauschii* AT428, doubled haploids Syn80 by colchicine application

Recombinant inbred lines (RILs):

Syn79 x Syn80, single seed desent from F_2 to F_9, a total of 230 SSDs
F9 RILs and their parents for genotyping, then the seeds of each F9 line in one block for trait evaluation.

Field trials

2 Each plot had five 1.5 m rows spaced 0.5 m apart. At the two-leaf stage, only ten evenly distributed plants in each row were retained for further growth. Field management consisted of commonly under-taken practices in wheat production.The trials were performed in randomized complete blocks with three replicates. A total of five trials were conducted at Guang-Han Station in 2017-2019 and Cang-Shan Station in 2017 and 2018.

Trait evaluation

3 In each plot, 10 plants with whole tissues including root and shoot were randomly selected, taken out from the field and put into a paper bags. When sampling, plants at the ends of each row were disregarded to minimize within-row edge effects. PH and TN were measured as the mean plant height and tiller number of 10 plants, respectively. And then the roots of 10 sampling plant were cut off and the SFW were measured as the mean shoot fresh weight of 10 plants. Once SFW was completed for the shoot of a plant, it were put in one drying oven at **120 °C** for 10 minutes and then dried to a constant weight for inveatigation of SDW at **65 °C** in another drying oven. All traits were described by the mean values of 10 plants for corresponding line. The measurement for PH, TN and SFW was accomplished within 12 hours.

SNP Genotyping

4 DNA extraction

A total of 50 mg of plant fresh leaves was collected from 2-week-old seedlings and DNA was extracted using the NuClean Plant Genomic DNA Kit (CWBio, Beijing, China). Eluted DNA was quantified using Qubit 4 Fluorometer (Life Technologies Holdings Pte Ltd, Singapore) and then normalized using a 12-channel electronic pipette with a volume range of 10 to 100μL (Eppendorf, Hamburg, Germany) to obtain the concentration required for genotyping.

5 SNP Array Analysis

being executed on the Affymetrix platform of Axiom Wheat Breeder's 15K SNP Genotyping Array by China Golden Marker Biotech Co Ltd (Beijing, China) http://www.cgmb.com.cn
The collected fluorescence signal from SNP array wereprocessedand analyzed by the functions of apt-genotype-axiom for genotype calling, ps-metrics for generating various QC metrics and ps-classification for classifying SNPs in the software of Affymetrix Axiom Analysis Suite version 4.0.1.

QTL analysis

6 Phenotypic analysis

Analysis of variance (ANOVA) and correlation analysis for the phenotypic data were calculated using the SPSS statistical package (SPSS Inc., Chicago, IL). https://www.ibm.com/products/spss-statistics
ANOVA was calculated based the general linear model (GLM). Broad sense heritability (H^2) was estimated with the formula: $H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge}/n + \sigma^2_e/nr)$, where $\sigma^2_g$is genetic variance, $\sigma^2_{ge}$is the variance of genotype-environment interaction, $\sigma^2_e$is experiment error variance, n is the number of trials, r is the number of replications

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7 The QTL IciMapping Software version 4.1 was used for genetic linkage map construction and QTL mapping.
http://www.isbreeding.net/software/?type=detail&id=18
The location of SNP marker was aligned according to the physical map of *Ae. tauschii* AL8/78 for D genome.

QTL analysis for the measured traits under the five different environmental conditions were performed using the inclusive composite interval mapping (ICIM) option. Significant LOD threshold was determined by 1000 permutations and P=0.05.

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