

hap3A.1 containing factors for resistance is a determinant of grain yield and stability performance in wheat

Introduction

Improving wheat grain yield (GY) and stability performance across multiple environments have become very crucial for global food security due to climatic change which accounts for one-third of crop yield variability (Ray *et al.*, 2015). The cultivation of high-yielding genotypes with stable performance is key towards decreasing yield gap and is desirable for crop management, marketing, cultivar recommendation, and profit making. Crop yield and stability traits are quantitatively (polygenic) inherited and are strongly influenced by genotype and environment interactions (GEI). Yield stability performance (YSP) may be related to its resistance or susceptibility to biotic and abiotic stress factors, resistance to pests and diseases, and tolerance to abiotic stresses. Duvick (1996) indicated that improved resistance or tolerance to any type of stress is essential for yield stability, suggesting that the simultaneous improvement of GY and YSP is possible via the incorporation of multiple factors for resistance and tolerance to stress into the cultivated germplasm. There are growing interests in using multi-environmental trials (METs) to explain the genetic factors of GEI and YSP in crops, e.g. rice (Hittalmani *et al.*, 2003) and barley (Malosetti *et al.*, 2004); but the genetic basis controlling both traits under different crop management systems (CMS) has not been investigated. In this study, we characterized 213 wheat cultivars [that have been released in the past 50 years] under three different CMS to: (i) understand the genetic diversity among them and the relationship between GY and YSP and (ii) use genome-wide association study (GWAS) to identify key genetic factors and candidate genes contributing to improve GY and YSP in wheat.

Materials and Methods

Germplasm evaluation

A total of 213 wheat diversity (BRIWECS) panel was evaluated at six diverse geographical locations in Germany in three seasons (2015-2017) under **LN_NF** (low-nitrogen, no-fungicides), **HN_NF** (high-nitrogen, no-fungicides), and **HN_WF** (high-nitrogen, +[plus] fungicides) CMS. The six locations include: **Bonn** (CKA), **Giessen** (GGE), **Giessen** (RHH), **Hannover** (HAN), **Quedlinburg** (QLB), and **Kiel**. Thus, the panel was evaluated in 54 different environments.



Figure 1. BRIWECS field trial in 2016 growing season at Bonn Klein-Altendorf during: seedling stage (A), ripening stage (B), and harvest (C).

Phenotypic Analysis

Grain yield METs data were analyzed for 2014/2015, 2015/2016, and 2016/2017 growing seasons.

Outliers were detected and removed

- o IQR method

ANOVA:

- o REML model linear model
- o Position effects: *Replication/Row*Column* [Gilmour *et al.*, 1995]
- o $H^2 = \frac{V_g}{[V_g + (V_{gt}/t) + (V_{gy}/y) + (V_{gty}/ty) + (V_{err/tyr})]}$

GY BLUES were estimated and used for:

- o Eco-valence stability indices (WESI)
- o Summary statistics
- o Scatter plots matrixes/Correlations
- o GGE biplots

$$WESI = [Y_{ij} - Y_i - Y_j - Y_{..}]^2 \text{ (Wricke, 1962)}$$

The $-\log_{10}$ transformed GY and WESI data were included as phenotypes in the GWAS analyses

Genetic Analyses

Genotyping was done using **135K** Axiom SNPs + **15K** Illumina SNPs

- o **48K** SNPs were obtained after cleaning
- o **GWAS** by MLM-P+K model
 - ✓ $y = X\beta + Sa + Qv + Zu + e$ (Yu *et al.*, 2006)
 - ✓ Significant associations were declared at $qFDR \leq 0.01$

- o **Identification of candidate genes co-segregating with the significant SNPs**

Results

Phenotypic analyses

ANOVA (REML) results (Table 1) showed significant ($P \leq 0.01$) genotype, CMS, and year effects on GY across the 6 locations; with $H^2 \geq 0.24 \leq 0.76$. The genotype-by-CMS interactions were also significant. As would be expected, wheat genotypes grown under **HN_NF** [except in QLB] and **HN_WF** increased GY by +5.47 to +24.32 % and +28.63 to +55.47 % across locations, respectively, when compared to **LN_NF** (Figure 2).

Table 1. ANOVA results for GY across locations under three CMS

Source	df	CKA	GGE	HAN	QLB	KIE	RHH
Gen	212	250**	4603**	8721**	1354**	1726**	441521**
CMS	2	53938**	352977**	675479**	276476**	197702.1**	47540733**
Yr	2	12183**	618236**	209906**	131800**	100971**	16533944**
Gen*CMS	424	24**	196**	567**	154**	83**	47038**
Gen*Yr	424	21**	370**	666**	189**	107**	96518**
CMS*Yr	4	2487**	32037**	19936**	51005**	8665**	360674**
Gen*CMS*Yr	846	6**	91**	163**	66**	40**	26771**
H^2		0.64	0.48	0.72	0.24	0.76	0.61
CV _{LN_NF}		17.53	22.89	22.27	22.20	16.87	26.37
CV _{HN_NF}		23.53	23.65	23.55	31.06	20.50	25.90
CV _{HN_WF}		11.74	14.01	16.35	27.53	12.51	11.11

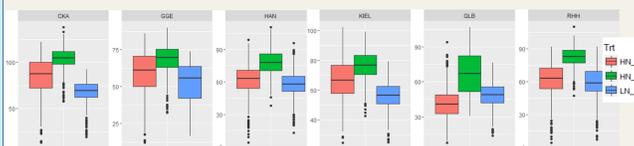


Figure 2. Boxplots showing distributions and genotypic performance of grain yield across locations under three different crop management systems

Yield Stability vis-à-vis Breeding progress

The genotypes exhibited significant variations in YSP (Figure 3 A). The stability of the genotypes were greatly improved (642.98) under **HN_WF**, but decreased (996.58) under **HN_NF** (Table 2). As shown in the scatter matrix plot (Figure 3B), genotypes included in the studied panel formed two distinct response pattern bases on their year of release. For instance, the newly released wheat genotypes (2004 – 2013) appeared to have higher GY and stability potentials than the older (1946-1977) ones under the evaluated.

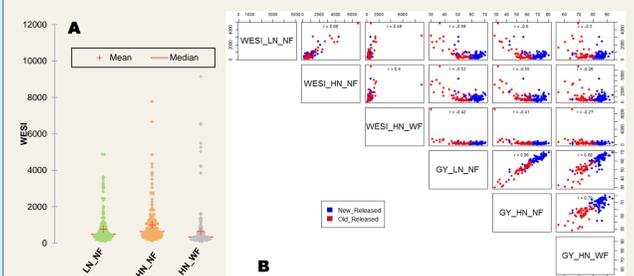


Figure 3. A. Scatter grams of grain yield stability (WESI) among 213 wheat genotypes under different CMS. B. Scatter matrix plots for new (blue) and old (red) wheat genotypes showing the relationship between grain yield and yield stability under different CMS

Table 2. Descriptive statistics of stability performance (WES) of wheat genotypes across 54 environments under LN_NF, HN_NF and HN_WF crop management systems

Parameters	LN_NF	HN_NF	HN_WF	Mean
WESI values	767.84	996.58	642.98	-
WESI_new released cultivars (2004-2013)	603.27	795.31	371.10	589.89
WESI_old released cultivars (1946-1977)	928.95	1284.64	817.26	1010.28
Gain over 50 yrs (%)	35.06	38.09	54.59	42.58
Gain per year (%)	+ 0.70	+ 0.76	+ 1.09	+0.85

Genome-wide association study

A total of 40 significant MTAs ($R^2 = 8.56$ to 16.82%) on 1B, 2B, 3A, and 7B were identified for GY and YSP. The detected SNPs on 3A are associated with both GY and YSP under **LN_NF** and **HN_NF** (Figure 4); while those on 1B and 7B affected YSP under **LN_NF/HN_NF** and **HN_WF**, respectively. The detected SNPs on 3A are in high-LD ($r^2 \geq 0.8$) at region spanning ~13.22 kb and overlapped 4 genes such as *CCB3*, *ABC*, *MPPA*, and *LRR1*. Moreover, the SNPs on Chr. 7B are co-localized with genes involved in phytotoxin resistance including *ASP*, *PER*, *HO1*, and *PLD*, suggesting that the loci may be responding to the applied fungicides and/or disease load.

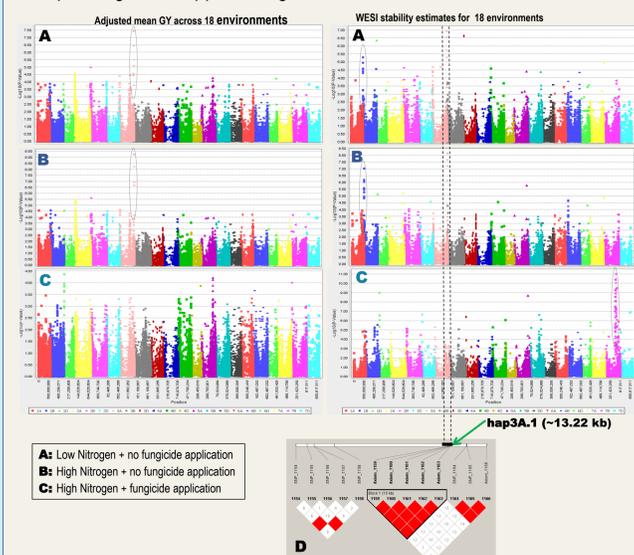


Figure 4. Manhattan plots for GY and yield stability based on 48,037 SNPs under (A) LN_NF (low N, - fungicides), (B) HN_NF (high N, - fungicides), and (C) HN_WF (high N, + fungicides) crop management systems. (D) Identified LD blocks based on pairwise r^2 values between SNPs on chromosome 3A.

Results (cont.)

Analysis of hap3A.1 region

Since the regional plots of associations (see Figure 4) in Chr. 3A (*hap3A.1*) region only showed significant signals for GY and YSP under CMS treated with low and high nitrogen with no fungicides, we suggest that the detected loci is involved in genotype-by-CMS interactions and may have something to do with plant defense. Among the four candidate genes co-located in the *hap3A.1* region is *LRR1* that is involved in plant defense. Chr. 3A has been reported to carry QTL for seedling & field APR (*QLr.fcu-3AL*, Chu *et al.*, 2009). But, because of lack of common markers, it is not known whether the *hap3A.1* is related to the QTL reported by Chu *et al.* (2009); thus warrant further molecular investigation before its application in MAS.

The haplotype analyses of *hap3A.1* showed that it contains two haplotypes: **-AACA-** and **-CCAC-**. Allelic effect of **-AACA-** significantly favored increased GY and improved YSP (Figure 6). Results showed that the new and old released wheat genotypes carrying the favourable **-AACA-** alleles tend to produce more grain yield and confer yield stability than those carrying the **-CCAC-** alleles (Figure 5C).

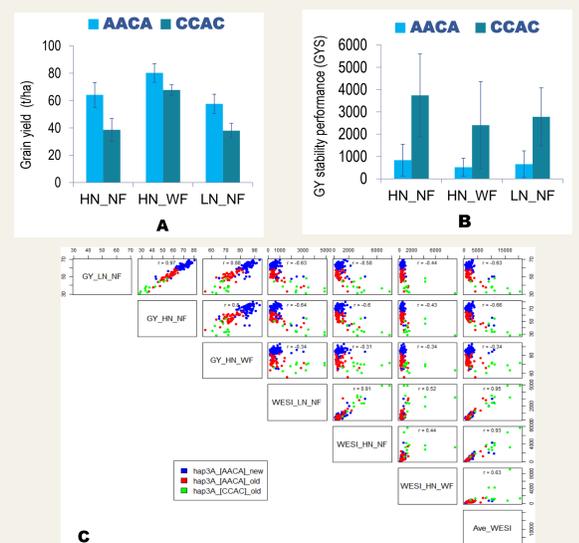


Figure 5. Allelic effects of *hap3A.1* on grain yield (A), grain yield stability (B), and individual wheat genotypes (C)

The stability of hap3A.1 across locations

GWAS were performed for the GY trait in each test environment over three years planting seasons under different CMS. Results indicated that the *hap3A.1* signal was present and/or commonly detected in 10, 14, and 1 test environments across locations under **LN_NF**, **HN_NF**, and **HN_WF**, respectively. This implies that *hap3A.1* for grain yield and yield stability performance is more likely to be less sensitive to the locational effect, but sensitive to G*CMS interactions that induced distinct reaction among the wheat genotypes.

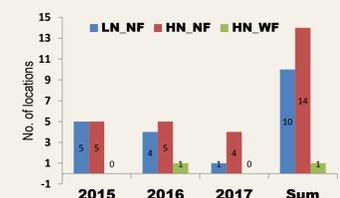


Figure 6. Bar plot of the total number of locations showing significant signals for *hap3A.1* under different CMS

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BRIWECS Partners

