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# Genomic regions influencing Preharvest Sprouting Tolerance in Two Doubled-Haploid Wheat Populations (*Triticum aestivum* L.)

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## Abstract

The current and projected climate change that is represented by increasing temperatures, humidity levels and irregular rainfall patterns, promotes the occurrence of preharvest sprouting (PHS) in wheat. PHS results in significant economic losses, globally, which necessitates the need for high-yielding cultivars with increased PHS tolerance, hence this study was conducted. The current study evaluated two doubled-haploid (DH) wheat populations of Tugela-Dn × Elands and Elands × Flamink across six environments in the Free State Province of South Africa to select genotypes with increased PHS tolerance and further map the underlying loci. Significant effects of DH lines (194) and environments (6) were observed for PHS tolerance. The results of this study validate previous findings that PHS is only expressed when environmental conditions are conducive. Quantitative trait loci (QTL) mapping using single nucleotide polymorphism (SNP) and silicoDArT markers revealed three additive QTL with major effects on chromosomes 5B and 7B, and these QTL were detected more than once, when conditions were favourable. These QTL explained a phenotypic variation (PVE) varying between 10.08% and 20.30% (LOD = 2.73 – 3.11). About 16.50% of DH lines performed to the level of Elands (the PHS-tolerant parent) and are



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recommended for further selection in a pre-breeding or breeding programme. The findings of the study are expected to facilitate the on-going breeding efforts for PHS tolerance in winter wheat.

**Keywords:** phenotypic selection, preharvest sprouting tolerance, QTL mapping analysis, SilicoDArT, SNP, wheat

## Introduction

Preharvest sprouting (PHS) is the premature germination of kernels on physiologically mature wheat ears upon continuous wet and humid conditions during the harvest season (Groos et al., 2002; Rodriguez et al., 2015). This phenomenon has resulted in significant reduction in wheat grain yield and end-use quality (Mares et al., 2005; Oluwaseyi et al., 2016; Martinez et al., 2018; Ali et al., 2019; Gupta et al., 2020). This is due to the activation of enzymes like lipases, amylases and proteases in developing kernels, which leads to the degradation of lipids, starch, and proteins (Andreoli et al., 2006; Simsek et al., 2014), and thus reduces the market value of wheat grain by up to 50% (Sorenson and Wiersma, 2004; Sorrells and Sherman, 2007; Gavazza et al., 2012; Martinez et al., 2018; Ali et al., 2019). In South Africa, a visual screening test of more than 2% sprouted kernels per 25 g wheat sample together with a falling number below 220 seconds are used to downgrade wheat to lower grades (Barnard, 2001; Craven et al., 2007). Annual grain yield and end-use quality losses due to PHS are estimated above 1 billion USD worldwide (Bewley et al., 2006; Depauw et al., 2012; Olaerts and Courtin, 2018; Ali et al., 2019).

A significant trait association has been reported between PHS tolerance and grain yield (Sharma et al., 1994; Singh et al., 2014). This makes PHS tolerance an important physiological trait in the improvement of wheat grain yield and end-use quality (Huang et al., 2006; Guzman et al., 2016; Nuttalla et al., 2017). Understanding the genetics of PHS tolerance in wheat can accelerate improvements of grain yield and quality, nonetheless, plant

adaptation remains vital in the overall plant performance (Kuzay et al., 2019). These improvements are needed to counteract South Africa's high annual wheat imports and the predicted crisis of global food insecurity (Mokone, 2017; Tadele, 2017; Kuzay et al., 2019).

Successful identification, validation and the application of loci underlying PHS tolerance have been reported in various bread wheat breeding programmes, globally, alongside other grain yield-related traits. These studies include Liu et al. (2013), Cabral et al. (2014), Gao et al. (2015), Lin et al. (2015), Cao et al. (2016), Su et al. (2016), El-Feki et al. (2018), Guan et al. (2018), Zeeshan et al. (2018), Wang et al. (2019), Gupta et al. (2020), Gautam et al. (2021) and He et al. (2021) among others. This has facilitated the improvement of wheat grain yield and end-use quality. The genetic diversity also exists for PHS tolerance in the South African wheat germplasm (Barnard, 2001; Barnard et al., 2005; Smit et al., 2010) and has been extensively utilized in wheat breeding programmes. However, the challenge remains to release high-yielding varieties with increased PHS tolerance, especially under the current and forecasted climate change (Nornberg et al., 2015).

The present study was conducted to evaluate PHS tolerance in bread wheat winter lines planted across multiple environments to select doubled-haploid (DH) lines with increased PHS tolerance and further map the underlying loci. The study objectives were to (1) examine the performance of DH lines and parents with regards to PHS tolerance across six environments; (2) identify quantitative trait loci (QTL) controlling PHS tolerance in the Tugela-Dn × Elands DH population; and (3) validate the presence of detected QTL in a population with different genetic background, Elands × Flamink.

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## Material and Methods

### Plant Material, Study Area and Experimental Design

Two DH wheat populations (n=210) derived from two crosses of Tugela-Dn × Elands (Lephuthing et al., 2021) and Elands × Flamink (Khumalo et al., 2021) and the three respective parents as checks (Tugela-Dn, Elands and Flamink) were provided by the Agricultural Research Council–Small Grain Germplasm Bank and evaluated for PHS tolerance. The study was conducted in six environments in the Free State Province of South Africa over two years, 2016 and 2017. Environments included Arlington 2016 (ARL1), Bethlehem 2016 (BHM3), Bethlehem 2017 (BHM4), Clarens 2016 (CLAR5), Harrismith 2016 (HAR7) and Harrismith 2017 (HAR8). Respective locations and weather descriptions of the six environments are shown in Table 1.

Parents were chosen because of their contrasting grain morphological characteristics, adaptability, as well as reactions to PHS tolerance (ARC, 1993, 1999). Tugela-Dn is a winter wheat cultivar with high yield potential and is extensively used for dry-land wheat production in South Africa. The cultivar, however, is highly susceptible to PHS. Elands is a facultative cultivar that was released for dry-land wheat production in 1998. The cultivar has high yield potential, excellent tolerance to PHS and exceptional bread-making quality, which makes it a quality standard in the South African wheat industry (Khumalo et al., 2021). Flamink is a winter wheat cultivar with high yield potential, however, exhibits a reduced level of tolerance to PHS. An augmented design was used in all environments as described in Khumalo et al. (2021). Commercial production and agronomic practices were followed as recommended for the specific production region.

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Table 1. Descriptions of the six study environments between grain filling, maturity, and harvest stages of wheat in 2016 and 2017 planting seasons.

| †Env | Locality | Period | Geographic position | | | Average Daily Temperature (°C) | | Average Daily Humidity (%) | | Average Daily Rainfall (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Longitude | Latitude | Altitude ǂ (m.a.s.l.) | Min | Max | Min | Max | |
| ARL1 | Arlington | October 2016 | 26.7732 | 28.0046 | 1435 | 12.67 | 27.00 | 28.33 | 48.67 | 1.66 |
| | | November 2016 | | | | 17.67 | 31.33 | 33.00 | 62.67 | 0.02 |
| | | December 2016 | | | | 18.67 | 31.67 | 30.00 | 52.00 | 0.03 |
| | | January 2017 | | | | 17.33 | 31.33 | 26.00 | 46.00 | 0.00 |
| BHM3 | Bethlehem | October 2016 | 28.2973 | -28.1628 | 1721 | 11.00 | 26.00 | 33.50 | 92.50 | 1.38 |
| | | November 2016 | | | | 14.05 | 27.26 | 35.25 | 94.40 | 4.01 |
| | | December 2016 | | | | 13.63 | 27.94 | 37.29 | 93.77 | 3.11 |
| | | January 2017 | | | | 13.32 | 26.20 | 41.65 | 94.53 | 4.56 |
| BHM4 | Bethlehem | October 2017 | 28.2973 | -28.1628 | 1721 | 7.06 | 24.61 | 27.28 | 90.13 | 1.39 |
| | | November 2017 | | | | 9.04 | 26.76 | 25.27 | 90.81 | 3.14 |
| | | December 2017 | | | | 12.23 | 26.66 | 35.84 | 93.01 | 3.69 |
| CLAR5 | Clarens | October 2016 | 28.5838 | -28.5038 | 1849 | 8.17 | 25.47 | 18.71 | 84.18 | 1.22 |
| | | November 2016 | | | | 11.37 | 25.38 | 34.19 | 92.64 | 3.45 |
| | | December 2016 | | | | 12.36 | 27.24 | 33.54 | 92.50 | 3.73 |
| | | January 2017 | | | | 12.30 | 25.00 | 41.07 | 93.13 | 3.90 |
| HAR7 | Harrismith | October 2016 | 29.11596 | -28.3128 | 1720 | 9.17 | 25.85 | 23.08 | 87.88 | 1.42 |
| | | November 2016 | | | | 12.09 | 25.42 | 42.99 | 91.43 | 4.43 |
| | | December 2016 | | | | 13.18 | 27.45 | 42.06 | 89.57 | 4.60 |
| | | January 2017 | | | | 12.66 | 26.58 | 47.29 | 89.68 | 5.54 |
| HAR8 | Harrismith | October 2017 | 29.11596 | -28.3128 | 1720 | 7.65 | 24.89 | 30.41 | 84.01 | 1.6 |
| | | November 2017 | | | | 9.76 | 27.05 | 28.93 | 80.81 | 2.73 |
| | | December 2017 | | | | 11.93 | 26.05 | 42.76 | 89.45 | 6.74 |

†Environment denotes ARL1 for Arlington 2016, BHM3 for Bethlehem 2016, BHM4 for Bethlehem 2017, CLAR5 for Clarens 2016, HAR7 for Harrismith 2016, and HAR8 for Harrismith 2017.

ǂm.a.s.l. denotes metre above sea level.

**Phenotypic Evaluation of PHS Tolerance**

At anthesis stage, 28 ears per DH line and parent were randomly tagged using insulation tape. As described by Barnard et al. (1997), the tagged wheat ears were hand harvested at physiological maturity, air-dried at room temperature, and stored in a cold room (4°C) to maintain dormancy until PHS evaluation. Wheat ears were then subjected to simulated rainfall in a humidified chamber at 15°C/25°C day/night temperature with 98% humidity for 72 hours and eventually scored for PHS tolerance according to a rating scale of 1 (not sprouted) – 8 (highly sprouted) (Figure 1; Barnard et al., 1997).

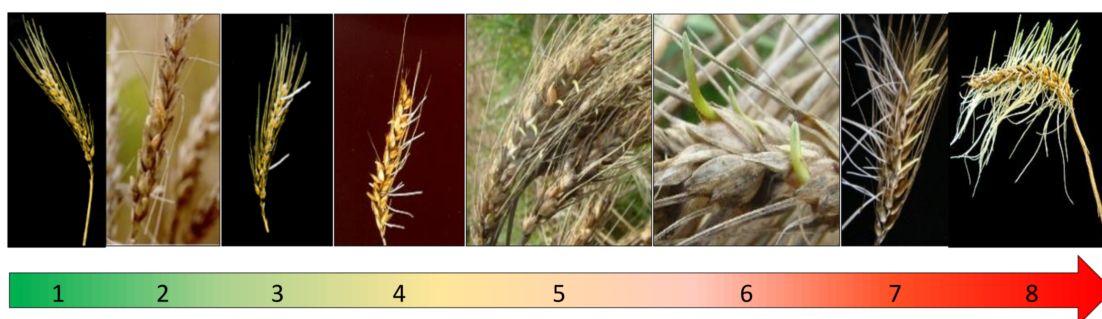


Figure 1. Rating scale (1 – 8) used to assign a tolerance or susceptible score to the studied material (Barnard et al., 1997). Scores 1 – 3 = tolerant; 4 – 5 = moderate; 6 – 8 = susceptible.

**Phenotypic Evaluation and Statistical Analysis**

Statistical analyses were performed on Genstat 18th Edition (VSN International, 2015) using 194 DH lines (139 Tugela-Dn ×Elands + 55 Elands × Flamink) following the removal of DH lines with missing data in most of the environments. Data was tested for normality and homogeneity of variance before conducting the analysis of variance (ANOVA). ANOVA was used to examine significant effects of genotypes, environments, and the genotype × environment interaction. The genotype × environment interaction was estimated from error mean square ($MS_{ge}$) of the replicated parents within environments according to an augmented design defined by Federer (1961). Patterns of genotype × environment interaction and genotype stability were illustrated on the additive main effects and multiplicative interaction

(AMMI) biplot and the frequency distribution of phenotypes in the six environments was depicted in histograms. The broad-sense heritability ($H^2$) estimate of PHS tolerance was calculated using the following formula (Huang et al., 2006; Tsilo et al., 2014):

$$1 - \frac{MSge}{MSg} \text{ or } \frac{\sigma^2 g}{[\sigma^2 g + \left(\frac{\sigma^2 ge}{e}\right) + (\frac{\sigma^2 e}{re})]}$$

Where $MS_{ge}$ and $MS_g$ represent the genotype × environment and the genotype mean squares respectively;

$\sigma^2 g$ is the genotypic variance = $(\frac{MSg - MSge}{re})$; $\sigma^2 ge$ is the genotype × environment interaction variance = $\frac{MSge - MSe}{r}$;

e and r represent the number of environments and replications, respectively;

and $\sigma^2 e$ is the error variance = $MS_e$.

## Genotyping and Construction of Genetic Map

The total genomic DNA was extracted from fresh leaves of three-weeks-old plants of 194 DH lines and three parents according to the Diversity Arrays Technology (DArT) plant DNA extraction protocol (http://www.diversityarrays.com/pub/DArT_DNA_isolation.pdf). The extracted DNA was genotyped with DArT-sequencing genotype-by-sequence (GBS) Platform 1.0 (DArT, Pty Ltd, Yarralumla, ACT, Australia), which produced 3204 single nucleotide polymorphism (SNP) and 9117 silicoDArT markers. Genotypic data were cleaned for redundant and non-polymorphic markers, markers with switched alleles, markers with ≥50% missing data, and significantly distorted markers ($p<0.05$) on RStudio version 1.1.463 (RStudio Team, 2019) and JoinMap® version 4.1 (van Ooijen, 2006). A total of 483 SNP and silicoDArT polymorphic markers were used to construct a genetic map for Tugela-Dn × Elands mapping population, while 1144 silicoDArT markers formed a genetic linkage map for Elands × Flamink mapping population. The order of markers within a linkage group was

established based on a regression mapping algorithm (Stam, 1993). Map distances (cM) were calculated from recombination frequencies using the Kosambi mapping function (Kosambi, 1943).

## QTL Analysis

QTL analysis was performed using Windows QTL Cartographer version 2.5 (Wang et al., 2012). The composite interval mapping (CIM) was used to screen for significant QTL using individual mean scores per environment and average mean scores across all environments. QTL detection was based on 1000 permutations ($\alpha$=0.05). The forward regression model was used with a window size of 10 cM, a walk speed of 2 cM, and five control markers. QTL were named following the international rules of genetic nomenclature adapted for wheat (McIntosh et al. 2003).

## Results

### Phenotypic Performance of Genotypes and Parents across Multi-Environments

The performance of genotypes in the six environments was depicted through histograms (frequency distribution) and AMMI biplot (genotype stability). ANOVA revealed significant differences among the three parents, one-hundred and ninety-four genotypes and six environments (Table 2). The genotype × environment interaction, which was estimated from error mean square (MSge) of replicated parents according to an augmented design defined by Federer (1961), was significant for PHS tolerance. Among the parents, Elands was PHS tolerant (score of 2.00), Flamink was moderately tolerant (score of 3.40), while Tugela-Dn displayed a susceptible reaction to PHS (score of 5.00) (Figure 2, Table 3). As expected, on average, the frequency distribution of genotypes was continuous (Figure 2), indicating the presence of transgressive segregation, with some individuals exhibiting higher or lower PHS

tolerance scores than the parents. This observation suggested polygenic control. A broad-sense heritability estimate of 0.5414 was calculated in DH lines (Table 2).

Two principal components of the AMMI biplot, PC1 = 36.16% and PC2 = 23.57% explained the observed phenotypic variation of genotypes across environments (Figure 3). Less genotype × environment interactions (indicated by the length of vectors) were observed in ARL1, BHM4 and CLAR5 in contrast to BHM3, HAR7 and HAR8. Most DH lines were found clustered toward the centre of the biplot, proving broad adaptation and good performance. The average performance of DH lines across environments followed the order HAR8 (most tolerant) > CLAR5 > BHM4 > HAR7 > ARL1 > BHM3 (least tolerant), with PHS tolerance scores ranging 3.00 – 4.00. These observations suggested tolerant to moderate reactions of the two DH populations. DH lines exhibited PHS tolerance scores ranging from 1.00 to 8.00, with an average score of 3.19±1.32. (mean±standard deviation).

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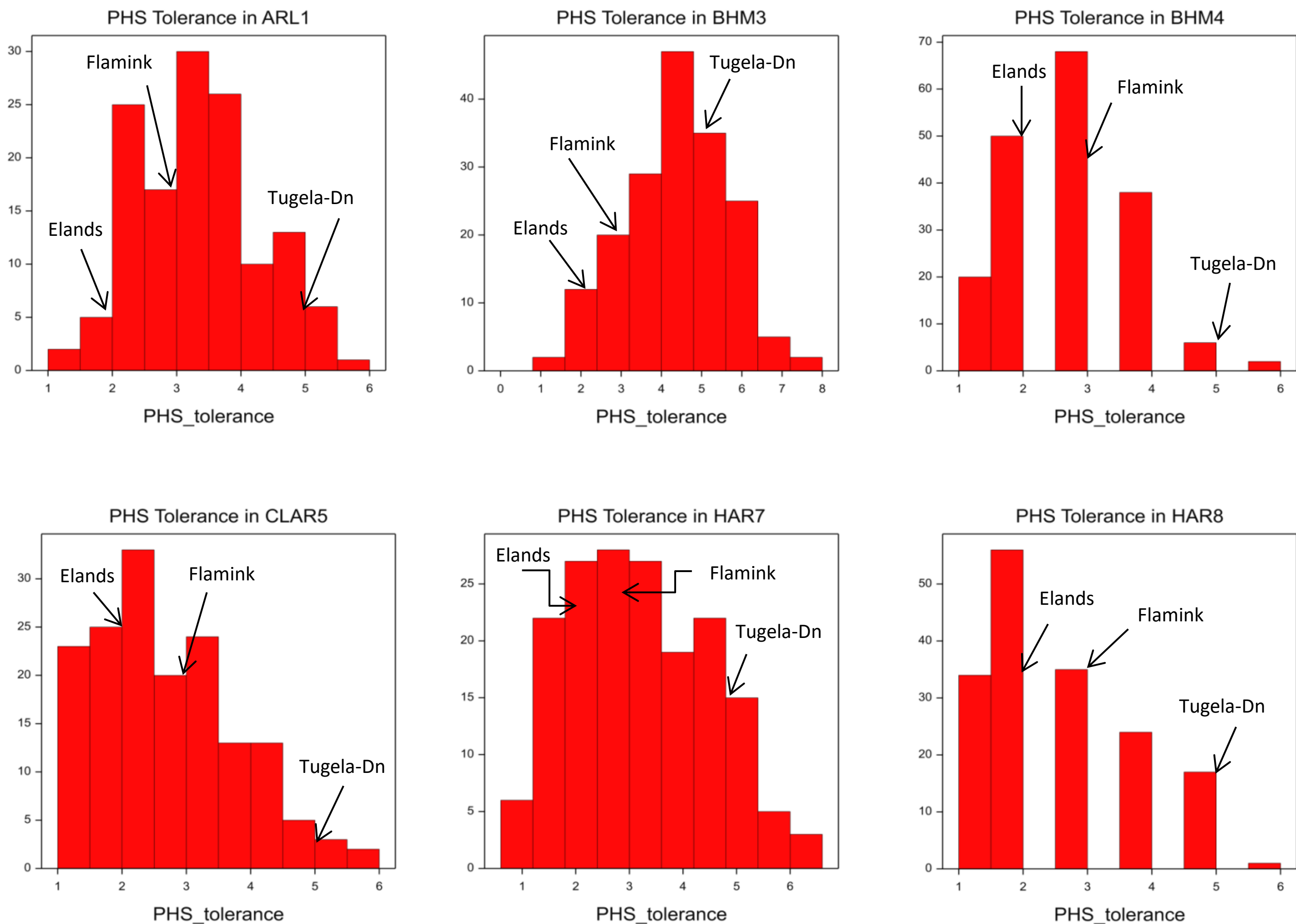


Figure 2. Frequency distribution of DH lines and parents for PHS tolerance in the six environments.

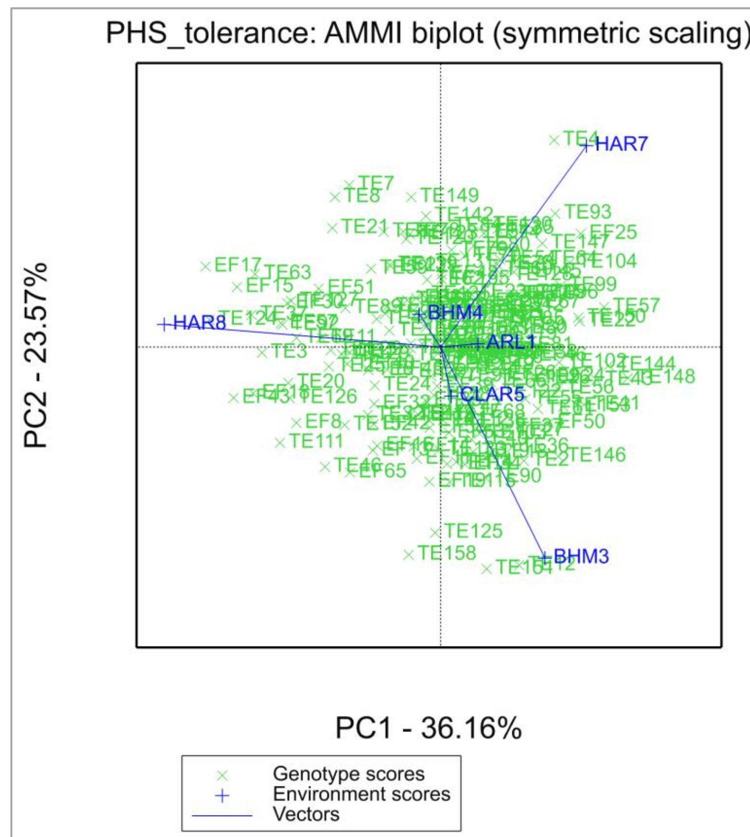


Figure 3. The AMMI biplot for PHS tolerance illustrating patterns of genotype × environment interaction and genotype stability across six environments.

Table 2. Mean squares and the broad-sense heritability ($H^2$) estimate of PHS tolerance in 194 DH lines and three parents across six environments.

| Source of variation | Degrees of Freedom | Mean Square | F (p-value) |
|---|---|---|---|
| Parents | 2 | 84.873 | <.001 |
| Environment | 5 | 5.434 | <.001 |
| Replications | 4 | 1.861 | |
| Parents × Environment | 9 | 1.580 | 0.017 |
| Residual | 56 | 0.627 | |
| | | | |
| Genotypes | 193 | 2.429 | <.001 |
| Environment | 5 | 74.182 | <.001 |
| Replications | | | |
| Genotype × Environment | 799 | 1.114 | - |
| $H^2$ | | 0.5414 | |
| $H^2$ (%) | | 54.14 | |

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**Selection of Best-performing Genotypes**

On average, most (62.37%) DH lines had PHS tolerance scores ≤3,00 (from a scale of 1 to 8, Figure 1), indicating tolerance of the two populations to PHS (Barnard et al., 1997). About 16.50% of DH lines performed to the level of Elands (the PHS-tolerant parent), with PHS tolerance scores ≤2.00 and are recommended for further selection in a pre-breeding or breeding programme. These DH lines are represented by the top ten best-performing genotypes in Table 3. Poorly performing DH lines similar to Tugela-Dn (the PHS-susceptible parent with a score of 5.00) are also represented by the top five worst-performing genotypes in Table 3.

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Table 3. PHS tolerance scores of DH lines represented by the top ten best- and the five worst-performing genotypes and scores of parents in the six environments and the average performance.

| †Genotype | ‡Environment | | | | | | Average PHS tolerance score |
|---|---|---|---|---|---|---|---|
| | ARL1 | BHM3 | BHM4 | CLAR5 | HAR7 | HAR8 | |
| PHS tolerance scores of the top ten best-performing DH lines | | | | | | | |
| EF 44 | * | 2 | 1 | 2 | 2 | 1 | 1 |
| EF 15 | * | 2 | 2 | 1 | 1 | 4 | 2 |
| EF 17 | * | 2 | 1 | 1 | 2 | 5 | 2 |
| EF 47 | * | 4 | 1 | 1 | 1 | 1 | 2 |
| TE_21 | 1 | 2 | 2 | 1 | 3 | 3 | 2 |
| TE_37 | 2 | 2 | 2 | 2 | 1 | 4 | 2 |
| TE_62 | 2 | 2 | 4 | 2 | 2 | 1 | 2 |
| TE 73 | 2 | 3 | 2 | 1 | 3 | 1 | 2 |
| TE 122 | 2 | 4 | 1 | 1 | 2 | 1 | 2 |
| TE 127 | 1 | 3 | 2 | 2 | 2 | 4 | 2 |
| PHS tolerance scores of the top five worst-performing DH lines | | | | | | | |
| TE 48 | 4 | 6 | 4 | 2 | 5 | 5 | 4 |
| TE 145 | 5 | 4 | 5 | 4 | 5 | 2 | 4 |
| TE 67 | 5 | 6 | 4 | 4 | 6 | 3 | 5 |
| TE 149 | 5 | 5 | 6 | 5 | 6 | 5 | 5 |
| TE 155 | 4 | 6 | 3 | 4 | 6 | 5 | 5 |
| Descriptive statistics | | | | | | | |
| Minimum | | | | 1.00 | | | |
| Maximum | | | | 8.00 | | | |
| Range | | | | 7.00 | | | |
| Mean | | | | 3.19 | | | |
| Standard deviation | | | | 1.32 | | | |
| Coefficient of variation | | | | 41.34 | | | |
| Parent | ‡Environment | | | | | | Average PHS tolerance score |
| | ARL1 | BHM3 | BHM4 | CLAR5 | HAR7 | HAR8 | |
| Tugela-Dn | 4 | 6 | 5 | 5 | 5 | 5 | 5 |
| Elands | 2 | 3 | 2 | 1 | 2 | 2 | 2 |
| Flamink | * | 5 | 3 | 3 | 4 | 2 | 3.4 |

†Genotype denotes a DH line. TE denotes a Tugela-Dn × Elands DH line, while EF denotes an Elands × Flamink DH line.

‡Environment denotes ARL1 for Arlington 2016, BHM3 for Bethlehem 2016, BHM4 for Bethlehem 2017, CLAR5 for Clarens 2016, HAR7 for Harrismith 2016, and HAR8 for Harrismith 2017.

AVE denotes the average PHS tolerance score across the six environments.

## Genetic Linkage Map Construction

The genetic map for the Tugela-Dn × Elands mapping population consisted of 483 polymorphic markers, which included an ALMT1-4 functional marker pair, 259 SNP and 223 silicoDArT markers. Twenty-three linkage groups (LG) were identified representing the 21

wheat chromosomes. The entire genetic map spanned 1516.57 cM of the wheat genome, with an average distance of 3.87 cM between adjacent markers (Table 4). Genetic distances between adjacent markers ranged from 0.58 cM on chromosome 6BLG1 to 8.71 cM on chromosome 3DLG2. The number of markers on each chromosome varied between seven on 3D.LG2 and 6D and 40 on 2B. More (51.76%) markers mapped to the B sub-genome, followed by the A sub-genome (24.84%), and the least number (23.40%) of markers was observed on the D sub-genome. The A, B and D sub-genomes covered total lengths of 480.77 cM, 588.92 cM, and 446.88 cM, respectively.

A total of 1144 polymorphic silicoDArT markers were used to construct the genetic map for Elands/Flamink mapping population. The genetic map represented all 21 wheat chromosomes and covered a length of 311.59 cM of the wheat genome, with an average distance of 0.27 cM between adjacent markers (Table 4). The number of markers on each chromosome varied between 11 on 4B and 83 on 2A. Chromosome 3D and 6D had the lowest marker density of 0.20 cM while 4B had the highest marker density of 1.21 cM. The A sub-genome was the longest with 107.59 cM (34.88% of markers), followed by the B sub-genome with 103.25 cM (29.02% of markers), and the shortest was the D sub-genome with 100.75 cM (36.10% of markers).

Molecular markers in the Elands × Flamink population had more data in comparison to the Tugela-Dn × Elands population, in which markers had high level of missing data and were, therefore, removed from analyses. As a result, denser linkage maps (with an average distance of 0.27 cM between adjacent markers) were formed with the Elands × Flamink mapping population in contrast to the Tugela-Dn × Elands (with an average distance of 3.87 cM between adjacent markers). Only 18 (silicoDArT) markers maintained the order between linkage maps of the two mapping populations. This amounted to 1.11% of the marker order similarity between the two mapping populations.

Table 4. Genetic linkage maps showing marker distribution in the 21 wheat chromosomes in Tugela-Dn × Elands and Elands × Flamink mapping populations.

| | Tugela-Dn × Elands linkage map | | | | Elands × Flamink linkage map | | | |
|---|---|---|---|---|---|---|---|---|
| LG† | Chromǂ | No. of markers | Map Length (cM) | Marker density (cM) | Chromǂ | No. of markers | Map Length (cM) | Marker density (cM) |
| 1 | Chr1A | 19 | 49.33 | 2.60 | Chr1A | 67 | 19.96 | 0.30 |
| 2 | Chr1B | 30 | 90.05 | 3.00 | Chr1B | 81 | 19.76 | 0.24 |
| 3 | Chr1D | 25 | 76.50 | 3.06 | Chr1D | 51 | 21.69 | 0.43 |
| 4 | Chr2A | 17 | 96.05 | 5.65 | Chr2A | 83 | 17.76 | 0.21 |
| 5 | Chr2B | 40 | 75.98 | 1.90 | Chr2B | 57 | 13.63 | 0.24 |
| 6 | Chr2D | 11 | 73.56 | 6.69 | Chr2D | 56 | 12.02 | 0.21 |
| 7 | Chr3A | 8 | 49.80 | 6.22 | Chr3A | 79 | 20.89 | 0.26 |
| 8 | Chr3B | 29 | 58.84 | 2.03 | Chr3B | 64 | 14.45 | 0.23 |
| 9 | Chr3D.LG1 | 8 | 28.95 | 3.62 | Chr3D | 59 | 11.84 | 0.20 |
| 10 | Chr3D.LG2 | 7 | 60.95 | 8.71 | Chr4A | 25 | 9.38 | 0.38 |
| 11 | Chr4A | 8 | 48.35 | 6.04 | Chr4B | 11 | 13.29 | 1.21 |
| 12 | Chr4B | 23 | 82.90 | 3.60 | Chr4D | 53 | 14.07 | 0.27 |
| 13 | Ch4D | 15 | 74.35 | 4.96 | Chr5A | 72 | 15.64 | 0.22 |
| 14 | Chr5A | 14 | 53.35 | 3.81 | Chr5B | 47 | 13.89 | 0.30 |
| 15 | Chr5B | 31 | 76.91 | 2.48 | Chr5D | 67 | 14.47 | 0.22 |
| 16 | Chr5D | 15 | 34.15 | 2.28 | Chr6A | 31 | 11.78 | 0.38 |
| 17 | Chr6A | 20 | 57.27 | 2.86 | Chr6B | 32 | 15.27 | 0.48 |
| 18 | Chr6B.LG1 | 31 | 17.90 | 0.58 | Chr6D | 59 | 11.61 | 0.20 |
| 19 | Chr6B.LG2 | 27 | 75.70 | 2.80 | Chr7A | 42 | 12.18 | 0.29 |
| 20 | Chr6D | 7 | 55.74 | 7.96 | Chr7B | 40 | 12.97 | 0.32 |
| 21 | Chr7A | 34 | 126.62 | 3.72 | Chr7D | 68 | 15.07 | 0.22 |
| 22 | Chr7B | 39 | 110.63 | 2.84 | | | | |
| 23 | Chr7D | 25 | 42.67 | 1.71 | | | | |
| | A sub-genome | 120 | 480.77 | 4.01 | A sub-genome | 399 | 107.59 | 0.27 |
| | B sub-genome | 250 | 588.92 | 2.36 | B sub-genome | 332 | 103.25 | 0.31 |
| | D sub-genome | 113 | 446.88 | 3.96 | D sub-genome | 413 | 100.75 | 0.24 |
| | Total Genome | 483 | 1516.57 | 3.87 | Total Genome | 1144 | 311.59 | 0.27 |

†LG denotes linkage group.

ǂChrom denotes chromosome.

## QTL Mapping Analysis

### Additive QTL Detected in the Tugela-Dn × Elands Mapping Population

A total of 14 additive QTL for PHS tolerance were detected across six environments in the Tugela-Dn × Elands mapping population (Table 5). Three QTL were detected in more than one environment and were considered to be stable. Stable QTL for PHS tolerance were

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identified on chromosomes 5B and 7B and explained a phenotypic variation (PVE) varying between 10.08% and 20.30% and had LOD scores ranging from 2.73 to 3.11. Elands (PHS-tolerant parent) contributed more (83.33%) additive effect than Tugela-Dn (PHS-susceptible parent) to the mapped stable QTL.

**Additive QTL Detected in the Elands × Flamink Mapping Population**

A single additive, however, unstable QTL for PHS tolerance was detected in HAR7 using the Elands × Flamink mapping population (Table 5). This QTL was mapped on chromosome 2D. Consequently, there were no putative stable QTL shared between the two genetic backgrounds.

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Table 5. Additive QTL for PHS tolerance detected in Tugela-Dn × Elands and Elands × Flamink DH mapping populations across six environments. QTL effects are only shown for environments with detected QTL.

| QTL mapped in the Tugela-Dn × Elands DH mapping population | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Nearby marker | Position[a] | QTL[b] | Detected environments[c] | | | | | | | QTL effects[d] | | | | | |
| | | | | ARL1 | BHM3 | BHM4 | CLAR5 | HAR7 | HAR8 | AVE | LOD | Add | PVE (%) | LOD | Add | PVE (%) |
| PHS | **4910940\|F\|0-22:A>G; 4395011\|F\|0-8:T>C** | **5B (28-29)** | ***QPhs.sgi-5B.3***$^{+}$ | **¥** | **¥** | √ | **¥** | **¥** | √ | **¥** | **3.11** | **0.33** | **10.08** | **3.01** | **-0.45** | **11.56** |
| | **5582828\|F\|0-6:C>T; 3024652\|F\|0-22:C>T** | **7B (60-66)** | ***QPhs.sgi-7B.4***$^{+}$ | **¥** | **¥** | **¥** | √ | √ | **¥** | **¥** | **3.10** | **-0.52** | **20.30** | **2.85** | **-0.49** | **11.89** |
| | **3021324\|F\|0-20:T>C; 6041508\|F\|0-33:G>T** | **7B (100-101)** | ***QPhs.sgi-7B.2***$^{+}$ | **¥** | √ | √ | **¥** | **¥** | **¥** | **¥** | **2.76** | **-0.48** | **10.66** | **2.73** | **-0.36** | **11.00** |
| | 7329308 | 7B (11) | *QPhs.sgi-7B.1* | ¥ | √ | ¥ | ¥ | ¥ | ¥ | ¥ | 3.21 | 0.51 | 10.97 | ¥ | ¥ | ¥ |
| | 3025468\|F\|0-18:T>G; 12343039\|F\|0-26:G>T | 3B (7) | *QPhs.sgi-3B*$^{+}$ | ¥ | ¥ | ¥ | √ | ¥ | ¥ | √ | 3.87 | -0.40 | 11.49 | 4.09 | -0.23 | 11.92 |
| | 4394765\|F\|0-8:C>G; 1684411\|F\|0-9:G>T | 7A (30-64) | *QPhs.sgi-7A*$^{+}$ | ¥ | ¥ | ¥ | ¥ | ¥ | √ | √ | 3.80 | 0.69 | 28.75 | 5.85 | 0.29 | 18.41 |
| | 5050436\|F\|0-32:T>C | 1A (20) | *QPhs.sgi-1A* | ¥ | ¥ | √ | ¥ | ¥ | ¥ | ¥ | 4.63 | -0.42 | 14.19 | ¥ | ¥ | ¥ |
| | 1082843\|F\|0-43:T>C | 1B (22) | *QPhs.sgi-1B* | ¥ | ¥ | ¥ | ¥ | √ | ¥ | ¥ | 2.56 | -0.53 | 16.97 | ¥ | ¥ | ¥ |
| | 5582507\|F\|0-13:C>G | 2A (90) | *QPhs.sgi-2A* | √ | ¥ | ¥ | ¥ | ¥ | ¥ | ¥ | 3.66 | -0.33 | 11.06 | ¥ | ¥ | ¥ |
| | 5324489; 5324039 | 2B (45-53) | *QPhs.sgi-2B.2* | ¥ | √ | ¥ | ¥ | ¥ | ¥ | ¥ | 2.92 | -0.49 | 8.15 | ¥ | ¥ | ¥ |
| | 3029334\|F\|0-25:C>G | 3DLG2 (4) | *QPhs.sgi-3DLG2* | ¥ | ¥ | ¥ | ¥ | ¥ | ¥ | √ | 2.52 | 0.27 | 16.03 | ¥ | ¥ | ¥ |
| | 4395594\|F\|0-22:T>C | 5B (38) | *QPhs.sgi-5B.1* | √ | ¥ | ¥ | ¥ | ¥ | ¥ | ¥ | 4.12 | 0.36 | 12.96 | ¥ | ¥ | ¥ |
| | 3064906\|F\|0-10:T>A | 5B (44) | *QPhs.sgi-5B.2* | √ | ¥ | ¥ | ¥ | ¥ | ¥ | ¥ | 3.78 | 0.47 | 22.63 | ¥ | ¥ | ¥ |
| | 1268172\|F\|0-33:C>G | 6B.LG2 (4) | *QPhs.sgi-6BLG2* | ¥ | √ | ¥ | ¥ | ¥ | ¥ | ¥ | 3.46 | -0.53 | 12.78 | ¥ | ¥ | ¥ |
| QTL mapped in the Elands × Flamink DH mapping population | | | | | | | | | | | | | | | | |
| PHS | 3029487 | 2D (0) | *QPhs.sgi-2D* | ¥ | ¥ | ¥ | ¥ | √ | ¥ | ¥ | 3.51 | -1.29 | 21.84 | ¥ | ¥ | ¥ |

[a]Position indicates the chromosome and exact position (in cM) in which the QTL was mapped.

[b]QTL were denoted according to McIntosh et al. (2003). QTL significance was tested at p=0.05, LOD = 2.5.

[c]Detected environments denotes ARL1, Arlington 2016; BHM3, Bethlehem 2016; BHM4, Bethlehem 2017; CLAR5, Clarens 2016; HAR7, Harrismith 2016; HAR8, Harrismith 2017; and "AVE" indicates the combined QTL analysis based on average PHS tolerance and TKW scores across all six environments.

[d]QTL effects describe the logarithm of the odds (LOD) score; the additive effect (Add); and the phenotypic variation explained by the QTL in percentage (PVE (%)). In the Tugela-Dn × Elands population, a negative additive effect shows that contributing alleles were from Elands and the positive effect indicates an influence by Tugela-Dn. In the Elands × Flamink population, a negative additive effect shows an influence by Elands while a positive effect indicates an influence by Flamink.

+ and bold indicate QTL shared between at least two environments.

## Discussion

### Phenotypic Variations Attributing to Environmental Differences

The results of transgressive segregation, the AMMI biplot and ANOVA revealed significant effects of genotypes, environments and the genotype × environment interaction for PHS tolerance. These phenotypic variations underscored the complex genetic control and the strong influence of environment on the expression of PHS tolerance (Mackay, 2004; Miles and Wayne, 2008; Kulwal et al., 2010; Marzougui et al., 2012; Barrero et al., 2015). Environmental factors such as temperature, relative humidity and rainfall received during the grain filling and maturation stages of wheat, greatly impact PHS tolerance (Gao et al., 2013; Mares and Mrva, 2014; Ali et al., 2019). The six study environments differed in average daily temperatures, humidity and rainfall received between grain filling, maturity, and harvest stages of wheat over the 2016 and 2017 planting seasons (Table 1). These could explain the observed variation in PHS response of genotypes (Figure 2, 3 and Table 3).

Cool temperatures (low humidity) retain seed dormancy whilst high temperatures during later stages of grain development can break embryo dormancy, thus increasing the chances of PHS if rain (more than 15 – 20 mm) occurs around harvest time (Mares, 1993; Biddulph et al., 2007; Mares and Mrva, 2014). Higher average daily humidity coupled with high average daily temperatures and rainfall were observed in BHM3 between grain filling and harvest stages of wheat (October 2016 – January 2017, Table 1). These conditions could explain the higher PHS scores (average of 4.40) recorded in this environment. On the contrary, ARL1 was the driest with high average daily temperatures, low average daily humidity and almost no rainfall and yielded moderately resistant PHS scores (average of 3.30).

The other environmental (CLAR5, HAR7 and BHM4) conditions were average and almost invariable with the average daily temperatures range of 24 – 27 °C, humidity of 84 – 93% and rainfall of 1.22 – 5.54 mm (Table 1). These environmental conditions are reportedly

favourable for growing winter wheat (DAFF, 2016), which could explain a good performance of genotypes observed in these environments in comparison to ARL1 and BHM3. These observations concur with the phenotypic analysis results which ranked the average performance of DH lines across environments in the following order: HAR8 (most tolerant) > CLAR5 > BHM4 > HAR7 > ARL1 > BHM3 (least tolerant).

Nonetheless, wheat producing regions in South Africa remain prone to PHS attributing to high temperatures coupled with summer rainfall that occur around the harvesting season (Sydenham and Barnard, 2018). This makes breeding for PHS tolerance a main target in these and other regions for the improvement of grain yield and quality (Huang et al., 2006; Guzman et al., 2016; Nuttalla et al., 2017). The results of the study indicated that the DH lines possess great phenotypic variation (ANOVA), stability across environments (AMMI biplot) and tolerance to PHS. These observations corroborated the QTL mapping of PHS tolerance to facilitate the improvement of wheat grain yield and end-use quality through marker assisted selection (MAS).

## QTL Mapping Analysis of PHS Tolerance

QTL detected in more than one of the six environments were considered stable and a PVE≥10% signified loci of major effect (Wang et al., 2018). Three stable additive QTL of major effect were detected for PHS tolerance through the Tugela-Dn × Elands mapping population (Table 5). These loci are population-specific (QTL × Genetic background interaction) as they could not be detected in the Elands × Flamink genetic background. Stable loci were mapped on chromosomes 5B (*QPhs.sgi-5B.3*$^{+}$) and 7B (*QPhs.sgi-7B.2*$^{+}$ and *QPhs.sgi-7B.4*$^{+}$). The well-known PHS-tolerant parent, Elands (ARC, 1999), was the main donor of favourable alleles in two (*QPhs.sgi-7B.2*$^{+}$ and *QPhs.sgi-7B.4*$^{+}$) of the three stable loci identified for PHS tolerance. Elands and the PHS-susceptible parent, Tugela-Dn, co-

influenced *QPhs.sgi-5B.3*$^{+}$. The effect of these loci was evident in the resistant to moderate phenotype that was observed across study environments (Figure 2, 3 and Table 3).

Our study results are comparable to the findings of other studies, which have reported >250 QTL associated with PHS tolerance on all 21 wheat chromosomes using diverse mapping populations (Gupta et al., 2020). Gupta et al. (2020) report that, up to date, there are 29 stable major QTL for PHS tolerance distributed on 12 different chromosomes, including 1B, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5A, 6A, 7B, and 7D. The findings of Gupta et al. (2020), Lin et al. (2016) and Martinez et al. (2018) are comparable to stable QTL for PHS tolerance identified on chromosome 7B in the present study. Cao et al. (2016) also reported a QTL conferring early heading, a trait indirectly influencing PHS tolerance on chromosome 7B, in the vicinity of the *Vrn-B3* locus. Singh et al. (2006) and Fofana et al. (2008) reported genomic regions associated with PHS resistance on chromosome 5B, which concur with the findings of the present study.

Other loci of major effects, however, detected only in single environments were identified on chromosomes 1A, 1D, 2D, 3A, 3B, 3D, 4A, 5A, 6B, and 7A, in the present study. The above-mentioned chromosomes and chromosome 2B have repeatedly been reported to harbour stable loci of minor and major effects for PHS tolerance in various studies. These include Roy et al. (1999), Groos et al. (2002), Mori et al. (2005), Munkvold et al. (2009), Kulwal et al. (2010), Cabral et al. (2014), Somyong et al. (2014), Lin et al. (2015, 2016), Zhou et al. (2017), and He et al. (2021) among others.

The QTL analysis results of our study attest that the inability to consistently detect stable QTL for PHS tolerance across all study environments does not necessarily mean that they are not present, but that the expression of the genotype's tolerance (favourable alleles) depends on many factors (Kulwal et al., 2010; Gao et al., 2013; Barrero et al., 2015). Firstly, the environmental variation (temperature, humidity, and rainfall), which its effect was found

significant ($p<.001$) in the present study, could modify the effects of alleles contributing to the genotype's tolerance to PHS (Mares and Mva, 2014; He et al., 2021).

Secondly, the expression of the genotype's tolerance to PHS is influenced by the genotype × environment interaction, which was found significant ($p=0.017$) in the present study, suggesting variable genetic effects in different environments (Rutter et al., 2006). Favourable alleles are not readily expressed until triggered by favourable weather (continuous rainy and humid) conditions prior to or during harvest (Groos et al., 2002; Rodriguez et al., 2015). Similarly, some weather conditions may suppress the expression and, therefore, the detection of PHS tolerance QTL. Lin et al. (2016) also observed inconsistency in the expression of stable QTL for PHS tolerance across eight environments, which included both greenhouse and field experiments. Lin et al. (2016) proved that the expression of other major stable QTL is suppressed in the presence of extremely high temperatures in the field. These findings are comparable to our results as diverse weather conditions were observed in the six study environments (Table 1).

Thirdly, the low broad-sense heritability ($H^2=54.14\%$), which was estimated from genotypes proved that more influence came from environmental factors influencing PHS tolerance. These and other factors significantly influence the expression and detection of PHS tolerance QTL across environments, which could explain inconsistencies observed with the detected stable QTL and their estimated QTL effects in the present study.

Mapping stable loci in different environments and over years (and even better in different genetic backgrounds) is crucial in MAS as it validates the presence, position, and the effect of that QTL (Hospital, 2009; Ogbonnaya et al., 2017). For example, locus *QPhs.sgi-7B.2*$^{+}$ that was mapped on chromosome 7B within 100-101 cM interval was detected from the same locality (Bethlehem) over two consecutive years (2016 and 2017). The two other stable loci mapped on chromosomes 5B (*QPhs.sgi-5B.3*$^{+}$) and 7B (*QPhs.sgi-7B.4*$^{+}$) were each detected

in the same year, but different localities. This proves the reliability of the three stable loci for PHS tolerance identified in the present study. A detected QTL may disappear after marker assisted introgression if it was a false positive or if its effect (expression) is highly influenced by either the QTL × QTL interaction, the QTL × Genetic background interaction, and/or the QTL × Environment interaction (Jannink et al., 2001; Shen et al., 2001). The three stable QTL identified for PHS tolerance have the potential to facilitate the improvement of PHS tolerance in winter wheat.

## Conclusions

The main aim of the present study was successfully executed. About 16.50% of DH lines performed to the level of Elands (the PHS-tolerant parent) and are recommended for further selection in a pre-breeding or breeding programme. Three stable major QTL controlling PHS tolerance were identified on chromosomes 5B and 7B in the Tugela-Dn × Elands genetic background. The two genetic backgrounds did not share QTL. The results of the study indicated that various factors affect the stability (detection and expression) of QTL for PHS tolerance. The findings of the study are expected to facilitate the improvement of PHS tolerance in winter wheat and provide a baseline for further validation of the detected loci.

## Author contribution

Conceptualization, T.P.K. and T.J.T.; methodology, T.P.K., T.H., A.B. and T.J.T.; formal analysis, T.P.K.; investigation, T.P.K.; writing—original draft preparation, T.P.K.; writing—review and editing, T.P.K., T.H., A.B. and T.J.T.; supervision, A.B. and T.J.T.; funding acquisition, T.J.T. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest**

"The authors declare no conflict of interest."

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