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# Climate Change Effects on a Diverse Set of Winter Wheat Evaluated Traditionally and With Machine Learning

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## ABSTRACT

Climate change is increasing the frequency and intensity of drought, which hampers wheat productivity from meeting the growing food demand worldwide. Therefore, improvements in yield under drought are urgently needed. This work evaluated a diverse set of 77 winter-wheat lines for two image-based early vigor traits and 15 mature traits of diverse winter-wheat lines. Early and late drought treatments were applied 12 and 65 days after vernalization, respectively. Further, a machine-learning-assisted phenotyping technique was adopted to measure spike area. Old Swedish cultivars showed the lowest early root vigor (4.92 cm) and large root biomass at maturity (5.25 g). No positive correlation was found between root biomass and yield components under the control condition. A high mean of grain yield was obtained in 1RS (9.8 g/plant), 2RL (9.5 g/plant), and cfAD99 (9.5 g/plant) genotypes under control. When including stability across control and two drought treatments, NGB, 1RS, 2RL, and cfAD99 genotypes showed the best performance. Peduncle length, root biomass, and NDVI positively contributed to the grain yield of 2RL genotypes under early drought, while 1000-grain weight and root biomass accounted for the high grain yield of 1RS genotypes under late drought. The image-based spike area measured by a machine-learning model correlated strongly to the yield component grain number ($R^2 = 0.70^{***}$). Furthermore, combined with yield reduction results, the spike area results suggested increased sterility (empty spikes) as the main cause of drought-induced yield loss instead of changes in spike size. Further integration of traditional measurements, modern phenotyping, and computational image analysis is needed to accelerate evaluations of plant traits under drought conditions. Genes potentially governing drought tolerance can be identified in these diverse lines.

## 1 | Introduction

Wheat (*Triticum aestivum* L.), one of the three major crops globally, is serving as the main protein and calorie source in the human daily diet (Shiferaw et al. 2013). Wheat is divided into spring and winter types, referring to the season when the crop is grown. In the northern hemisphere, the winter type is sown in the autumn and needs vernalization during the winter before it can set flowers and produce seeds (Crofts 1989). Generally, winter wheat is advantageous to spring wheat in regard to yield performance and tolerance to abiotic and biotic stresses (Afzal et al. 2015; Entz and Fowler 1991). In Sweden, according to the latest statistics (2024), the area of harvested winter wheat (413,830 ha) is almost seven times that of spring wheat (61,190 ha), giving approximately 12 times difference in total production between winter (2,708,400 t) and spring wheat (222,200 t; https://jordbruksverket.se/statistik; accessed on 6th May 2025). With the predicted climate change, wheat yield is expected to fluctuate largely due to increased levels of abiotic stress, such as drought and heat (Langridge and Reynolds 2021). Thus, the development of winter wheat genotypes tolerant to the predicted abiotic stress is of great economic and strategic importance.

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As the most important and representative abiotic stress, drought is closely linked to climate change (Dai et al. 2018). The geographical distribution, duration, and intensity of drought events are increasing due to the rising global temperature (Cook et al. 2018). Thus, the negative impact of drought on the production of cereal crops is increasing, making this abiotic stress a primary driver of yield loss (Johansson et al. 2023; Langridge and Reynolds 2021). The lack of water inhibits plant growth in different aspects and to varying degrees. Drought stress at vegetative growth impairs (morphologically and physiologically) the development of different organs, while the reproductive-stage drought reduces (biochemically and physiologically) the final yield severely (Johansson et al. 2023; Lan et al. 2022). Significant reductions in grain yield, 1000-grain weight, grains per spike, and spike length have been recorded in winter lines exposed to summer drought (Dickin and Wright 2008). In addition to field trials, the grain yield studied under controlled conditions has been proven to capture crop variations in unit productivity, which closely reflects drought tolerance (Alvarez-Morezuelas et al. 2022; Lama et al. 2023). Various studies have shown that different yield-related traits, for example, flag leaf area (Foulkes et al. 2007) and root biomass (Fang et al. 2017), are associated with drought tolerance in winter wheat.

Winter wheat of different genetic origins has been evaluated for its tolerance to drought under various environmental conditions (Fang et al. 2017, 2011; Wang et al. 2016). In these studies, some old cultivars, landraces, and primitive wheat have demonstrated performances that indicate their potential resilience to varying climates, although modern genotypes generally have shown better yield performance (Fufa et al. 2005). Also, wheat-alien introgression lines have been shown to contain genes that might contribute to tolerance to biotic and abiotic stresses. In such studies, the rye chromosomes 3R, 5R, and 7R have been reported to carry genes responsible for drought tolerance (Mohammadi et al. 2003). Additionally, winter wheat introgression lines containing chromosome 1RS have been found to contribute to drought tolerance by increasing the root biomass (Hoffmann 2008). However, combinations of various rye introgressions from different chromosomes have been limitedly investigated.

The present study is built on the hypothesis that a sufficiently diverse winter wheat material collection contains genes of superior performance under drought stress, and via accurate trait investigations, it should be possible to identify genetic backgrounds that hold traits of relevance for drought tolerance. Thus, this study aimed to evaluate the drought tolerance of winter lines from diverse sources by characterizing the 15 phenotypic traits of these genotypes grown under drought. The responses to drought on each trait were broken down into 13 genotype groups, attempting to identify genetic backgrounds that likely contain genes controlling drought tolerance in winter wheat.

## 2 | Materials and Methods

### 2.1 | Plant Materials

A total of 77 winter wheat (*Triticum aestivum*) genotypes representing a wide variation of genetic resources that is, three modern breeding lines provided by the company Lantmännen, 11 old Swedish cultivars, seven old lines provided by the Nordic genetic resource center NordGen, 46 wheat-rye introgression lines carrying chromosome 1R ($n=12$), 1RL ($n=1$), 1R+2R ($n=1$), 1R+6R ($n=8$), 1RS ($n=3$), 1RS+2RL ($n=3$), 2RL ($n=4$), 5R ($n=2$), 5RS ($n=7$), 5RS+4R+6R ($n=4$), and unknown ($n=1$) in the form of translocation and substitution, six introgression lines crossed from wheat-*Leymus mollis* line AD99, two triticale parental lines, and two Swedish traditional wheat parental lines were used in this study (Table S1). Detailed information for genotypes of each genotype group is presented in Table S2. The introgression genotypes with 1RL, 1R+2R, 5R, and unknown chromosomes were not included in mean comparison due to the small number of lines. The data of these lines were included in ANOVA, correlation analysis, PCA, and stability analysis.

### 2.2 | Early Vigor Assay

A greenhouse hydroponic experiment, following the procedure described by Lan et al. (2022) was conducted to evaluate the early vigor of roots, measured as digital root length (DRL). Seeds of different winter genotypes were first germinated on filter paper at a low-temperature condition (4°C, 48 h) for uniform sprouting, and then transferred to dedicated blue blotter paper (210 mm × 297 mm, Anchor paper company) that was dipped in water for consistent wetness. After 7 days of growth at 25°C/18°C, day/night, root length of four biological replicates (two plants in each replicate) was phenotyped digitally by a digital single-lens reflex (DSLR) camera (Canon 1300D, Canon Inc., Tokyo, Japan) using a previously described phenotyping technique (Kumar et al. 2020; Lan et al. 2022).

The early vigor of shoots, measured as digital leaf area (DLA) was phenotyped from three angles (Armonienė et al. 2018) using two DSLR cameras (Canon 1300D, Canon Inc., Tokyo, Japan) of three biological replicates (three plants in each replicate) of plants grown in Biotron (vernalization and growing conditions described below) 76 days after sowing (7 days after a 10-day germination and a 2-month vernalization period).

Cameras for both DRL and DLA phenotyping were operated by digiCamControl (http://digicamcontrol.com/, accessed on 25 May 2023), while DRL and DLA data were extracted from raw images by RootNav (Pound et al. 2013) and Easy Leaf Area (https://www.quantitative-plant.org/software/easy-leaf-area, accessed on 9 May 2025), respectively.

### 2.3 | Biotron Experiment

The biotron experiment was conducted from January to September 2021 in the Biotron at the Swedish University of Agricultural Sciences in Alnarp, Sweden. Seeds of three biological replicates for 78 genotypes in each treatment (treatments described below) were sown in 2.5 L plastic pots filled with peat-based soil placed in an artificial light climate (KK) chamber at a temperature-humidity condition of 18°C–70%. After germination, a two-month vernalization condition was initiated in the KK chamber at 4°C of temperature, 70% of humidity, 250 mol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$ of light intensity, and 8 h of day length. After the vernalization, all plants were transferred to a daylight (DK) chamber to obtain a natural light condition, while the temperature and humidity were hourly regulated according to a 10-year (2010–2019) mean weather data of Malmö obtained from the Swedish Meteorological and Hydrological Institute (SMHI). Details for the climate parameters utilized follow those of a previous study (Lan et al. 2022). Plants of three biological replicates were subjected to three growing conditions, that is, the control growing conditions (C) representing a water frequency of every second day until yellowish spike, early-drought-stress (EDS) conditions where a 28-day water withholding was applied from 12 days after vernalization (Zadok's 23) with the same water frequency as C conditions for the rest of the growing period, and late-drought-stress (LDS) conditions standing for a 14-day water withholding from 65 days after vernalization (Zadok's 50) with a C-condition water strategy for the rest of the growing period.

## 2.4 | Morphological, Physiological, and Yield Traits

Days to heading (DTH; number of days from sowing to the appearance of spikes) and anthesis (DTA; number of days from sowing to the appearance of anthers) were recorded manually. Normalized difference vegetation index (NDVI) was measured by a portable NDVI meter. Spike length (SPL; excluding awn length) and peduncle length (PDL; length from flag leaf to base of spike) were measured with a ruler in centimeters. Tillers per plant (TPP), spikes per plant (SPP), productive spikes per plant (PSPP), grains per plant (GPP), and grains per spike (GPS) were counted. Flag-leaf area (FLA) was measured with an LI-3000C Portable Leaf Area Meter (LI-COR Environmental, USA). Grain weight per plant (GWPP; grain yield in this study), grain weight per spike (GWPS), 1000-grain weight (TGW) (CONTADOR seed counter, Pfeuffer GmbH, Kitzingen, Germany) and dry root biomass (RB) were measured in grams.

## 2.5 | Digital Spike Area Trait

Spike area per plant (SAPP) was extracted from images (.jpg) using trainable Weka (Waikato Environment for Knowledge Analysis) segmentation (TWS) in ImageJ (https://imagej.net/ij/; version: 1.54f; accessed on 6th May 2025). As described in Figure 1, 15 images (5 control + 5 EDS + 5 LDS) were randomly selected to train a machine-learning model by manually defining the class of 'spike' and 'background' in TWS, and each training process was repeated with minor corrections on misclassified pixels until clear distinguishment between the two classes. The model was then applied to 682 (227 control + 228 EDS + 227 LDS) spike images for automatic spike detection. The spike-detected images were converted to 8-bit format to remove the background for the final spike area measurement,

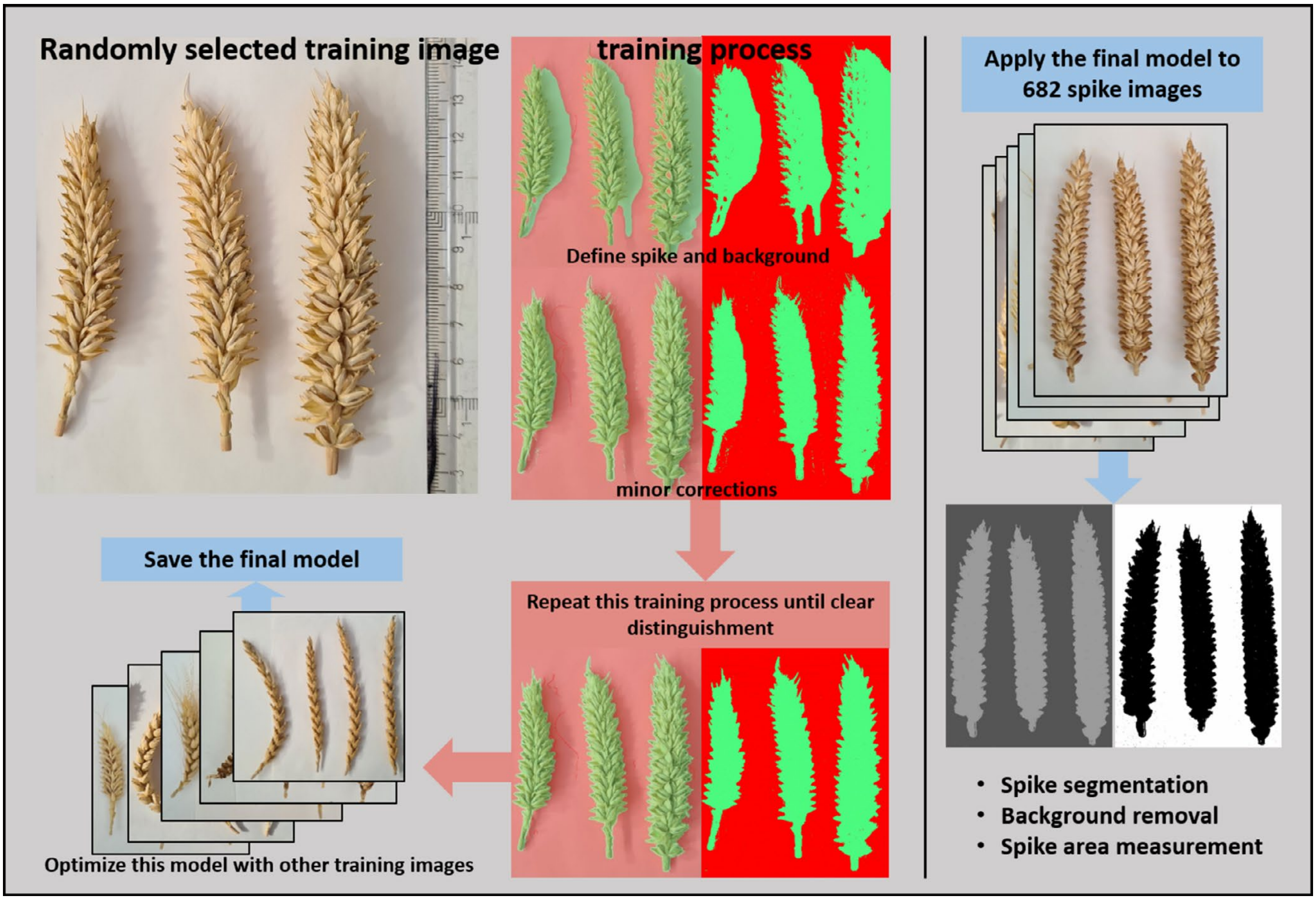

**FIGURE 1** | The training procedure for the machine-learning model trained in trainable Weka segmentation and spike area measurement performed in ImageJ.

and a macro script was written to automate this step in ImageJ (Appendix A).

### 2.6 | Data Analysis

The data of all the studied traits can be found in Table S3. All statistical analyses were performed using software Rstudio Team (2015), version 2023.03.1 + 446. A two-way ANOVA was performed to study the effects of genotypes, treatments, and genotype-treatment interactions, while mean comparisons were conducted using Tukey's posthoc test for variations between genotype groups and effects of treatments on each genotype group using R package "agricolae." The morphology-yield correlation was analyzed using Pearson's correlation coefficients in R packages "Hmisc" and "corrplot." Principal component analysis (PCA) was performed with the package "ggfortify" to explore the trait-treatment relationship while the additive main effect and multiplicative interaction (AMMI) was conducted with R package'meta' to identify individual genotypes with high and stable performance.

## 3 | Results

### 3.1 | Early Root and Shoot Vigor

Significant variations (one-way ANOVA, $p < 0.001$) in both digital root length (DRL) and digital leaf area (DLA) were found among evaluated genotypes (Table S4), ranging from 1.79 cm to 20.5 cm in DRL and from 11.0 $cm^2$ to 83.5 $cm^2$ in DLA. The lack of correlation (result not shown) between DRL and DLA suggested separate genetic mechanisms responsible for the early vigor of root and shoot development.

Clear significant variations were noted for DRL among genotype groups with the long roots in NGB genotypes (16.9 cm) and triticale parental lines (17.0 cm) and the significantly shortest roots in old genotypes (4.92 cm; Figure 2A). A significantly higher DLA was found in TPL (69.5 $cm^2$) as compared to 1R + 6R (44.4 $cm^2$), 5RS + 4R + 6R (42.4 $cm^2$), and WPL (41.1 $cm^2$) genotype groups, while no significant difference was noted for the rest of the genotype groups for DLA (Figure 2B).

### 3.2 | Genetic Variations and Drought Effects

The genotype groups were differently affected by the drought conditions applied in the present study (Figures 3 and S1). For morphological traits, the NGB genotype group showed a significantly higher FLA (56.2 $cm^2$) than most of the other genotype groups under C, and the FLA was also significantly the highest for these genotypes under EDS (58.3 $cm^2$). The LDS reduced FLA in genotype groups with a high level under C (e.g., modern and NGB) resulting in most genotype groups not differing significantly in regard to FLA (Figure 3A). High and low PDL were found in TPL (35.4 cm) and 1RS + 2RL (13.3 cm) genotypes under C, respectively. LDS showed severe inhibition on PDL of most genotype groups (Figure S1A). The RB was significantly higher under C in old (5.25 g), 1R (4.6 g), and 1RS + 2RL (6.22 g) genotypes than in modern (2.43 g), NGB (2.54 g), TPL (2.09 g), and WPL (1.97 g) genotypes. Both EDS and LDS resulted in a decreased RB within the genotype groups that showed a high RB under C (Figure 3B), resulting in mostly non-significant differences among genotype groups. 1RS + 2RL (8) genotypes showed high TPP, and most genotype groups showed increased TPP under LDS, with the exception of modern genotypes and those with 1RS + 2RL and 5RS + 4R + 6R (Figure S1B).

For yield traits, SPP and PSPP showed values essentially ranging from 3 to 5 under C while both EDS and LDS significantly and negatively affected old, 1R (PSPP not affected by EDS), 1R + 6R, and 2RL genotypes (Figure S1C,D). High SPL under C was found in 2RL (12.2 cm), 5RS (11.7 cm), and TPL (12.3 cm) genotypes. EDS and LDS decreased SPL in most genotype

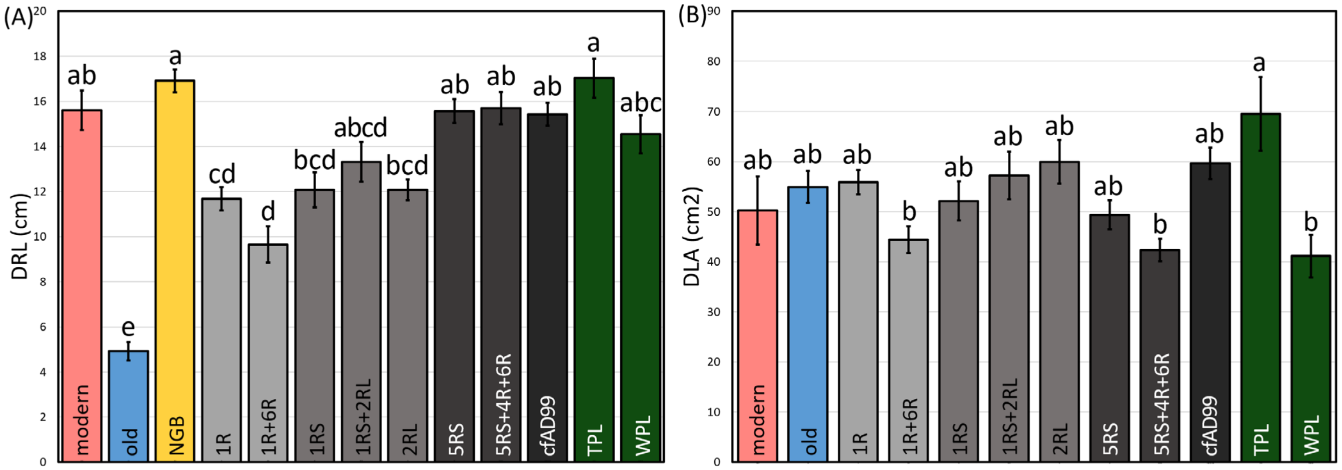

**FIGURE 2** | Comparisons of early vigor measured as (A) digital root length (DRL) and (B) digital leaf area (DLA) in winter-wheat lines of different genetic backgrounds, including modern: Modern cultivars and breeding lines received from company Lantmännen, old: Old Swedish lines, NGB: Cultivars received from the gene bank NordGen, introgression lines carrying rye chromosome 1R, 1R + 6R, 1RS, 1RS + 2RL, 2RL, 5RS, 5RS + 4R + 6R, cfAD99: Introgression lines crossed from wheat-*Leymus mollis* hybrids AD99, TPL and WPL: Triticale and wheat parental lines for wheat-rye introgressions. Mean values significantly differing between genotype groups at $p < 0.05$ (Tukey's posthoc test) are indicated by different compact letters. NGB genotypes are not included for DLA due to their late sowing time.

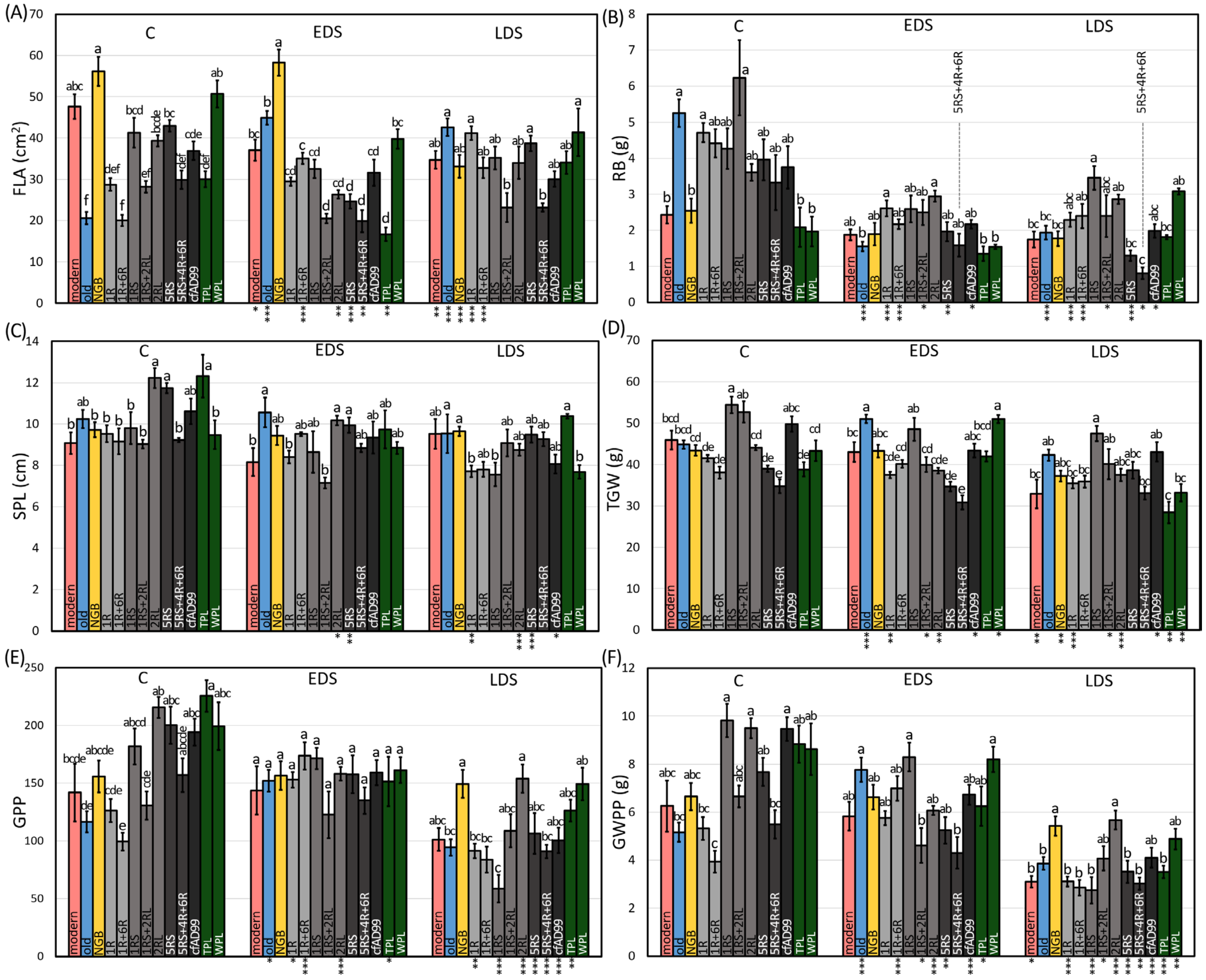

**FIGURE 3** | The mean (A) flag-leaf area, (B) root biomass (RB), (C) spike length (SPL), (D) 1000-grain weight (TGW), (E) grains per plant (GPP), and (F) grain weight per plant (GWPP) in winter-wheat lines of different genetic backgrounds, including modern: Modern cultivars and breeding lines received from company Lantmännen; old: Old Swedish lines; NGB: Cultivars received from gene bank NordGen; introgression lines carrying rye chromosome 1R, 1R + 6R, 1RS, 1RS + 2RL, 2RL, 5RS, 5RS + 4R + 6R; cfAD99: Introgression lines crossed from wheat-*Leymus mollis* hybrids AD99; TPL and WPL: Triticale and wheat parental lines for wheat-rye introgressions. C, control; EDS, early drought stress; and LDS, late drought stress. Mean values significantly differing between genotype groups at $p < 0.05$ (Tukey's post hoc test) are indicated by different compact letters. Each genotype group that differs between C and EDS/LDS is indicated by stars at ***: Sig. < 0.001, **: Sig. < 0.01, *: Sig. < 0.05 (Dunnett's test).

groups, although some of the genotype groups were not significantly affected (Figure 3C). LDS displayed a more severe impact on yield traits than EDS, with the reduction percentage of GPP and GWPP ranging from 4.1%–63.9% to 18.4%–68.7%, respectively, under LDS (Table S5). The grain size (TGW), reduced by 0.9%–28.5%, was less severely affected by LDS compared to grain number and grain weight traits (Table S5). 1RS genotypes group showed a significantly higher TGW (54.4 g) than the majority of the rest of the genotype groups under C, and a high TGW of 1RS genotypes was well sustained across EDS and LDS (Figure 3D). TPL genotypes were found with high grain numbers (GPP and GPS) under C, and for the majority of the genotype groups, LDS caused larger impacts on both GPP and GPS than EDS (Figures 3E and S1E). 1RS, 2RL, and cfAD99 genotypes showed a high level of GWPP and GWPS under C, while the lowest GWPP (3.93 g) and GWPS (1.27 g) were both found in 1R + 6R genotypes under C. Similar to grain number traits, more genotype groups were affected more severely by LDS than by EDS (Figures 3F and S1F).

## 3.3 | Treatment-Trait and Inter-Trait Relationships

From the principal component analysis (PCA) with 15 studied traits (DTH, DTA, NDVI, FLA, PDL, RB, TPP, SPP, PSPP, SPL, GPP, GPS, GWPP, GWPS, and TGW), samples of different drought treatments were divided along the first principal component (PC1) axis, explaining 31.73% of the overall variation (Figure 4A), which verified the significant effects of drought observed by a two-way ANOVA (Table S6). Control (C) and LDS samples were mainly located with both positive and negative values on PC1, respectively, indicating higher RB, NDVI, PDL,

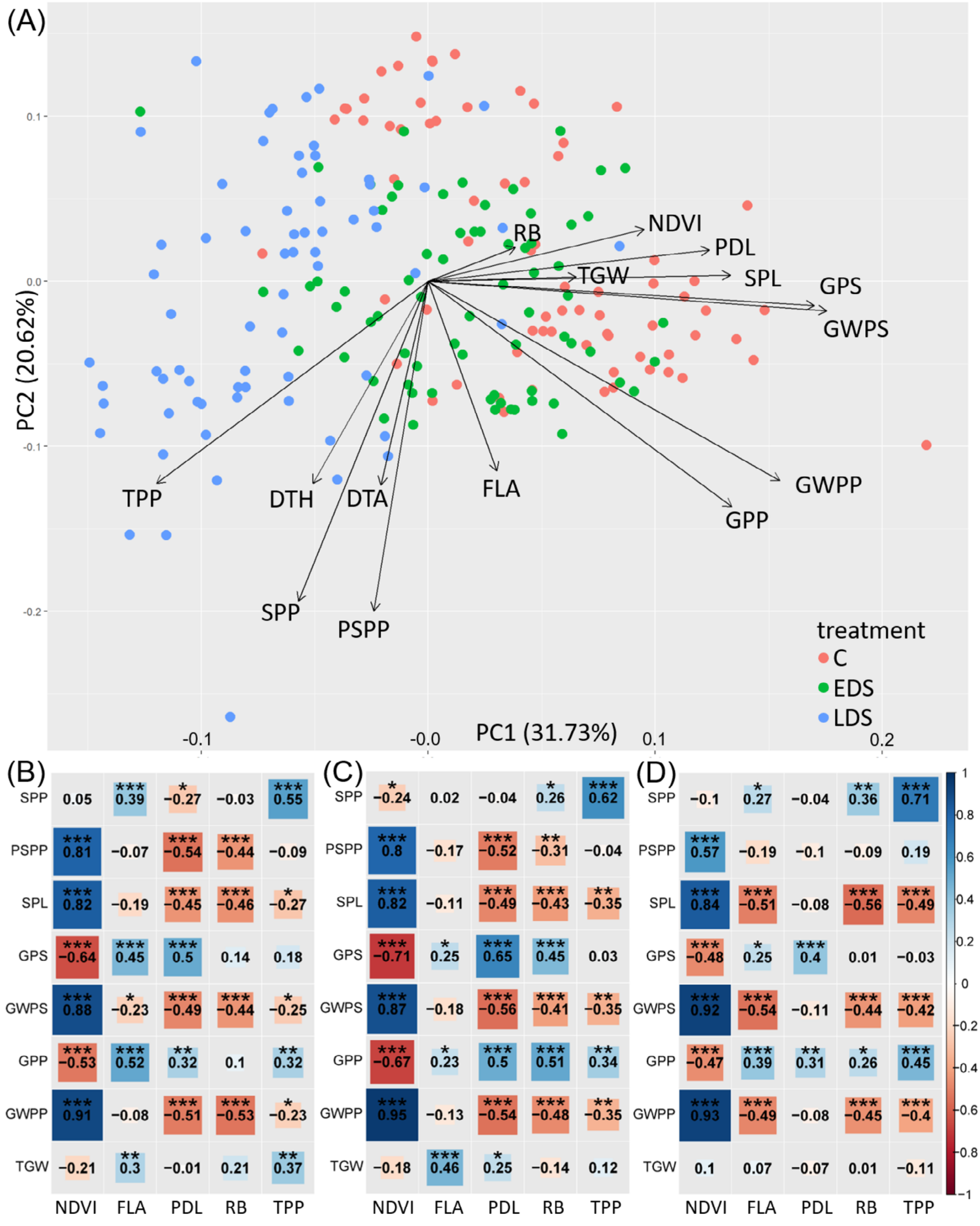

**FIGURE 4** | The treatment-trait relationship analyzed by (A) Biplot from principal-component analysis (PCA) for days to heading (DTH), days to anthesis (DTA), normalized difference vegetation index (NDVI), flag-leaf area (FLA), peduncle length (PDL), root biomass (RB), tillers per plant (TPP), spikes per plant (SPP), productive spikes per plant (PSPP), spike length (SPL), grains per plant (GPP), grains per spike (GPS), grain weight per plant (GWPP), grain weight per spike (GWPS), and 1000-grain weight (TGW) in 78 winter-wheat genotypes under control (C), early drought stress (EDS), and late drought stress (LDS), and the inter-trait (morphological and physiological traits versus yield traits) relationship under (B) control, (C) EDS, and (D) LDS analyzed by correlation analysis using Pearson's correlation coefficient. ***: Sig. < 0.001, **: Sig. < 0.01, *: Sig. < 0.05.

TGW, SPL, GPS, GWPS, GWPP, and GPP of C samples than those of LDS samples. The second principal component (PC2), explaining 20.62% of the variation, differentiated the sample performance in traits DTH, DTA, SPP, PSPP, and FLA independent of drought stresses (Figure 4A).

Several significant correlations were shown between yield (SPP, PSPP, SPL, GPS, GWPS, GPP, GWPP, and TGW) and non-yield traits (NDVI, FLA, PDL, RB, and TPP) parameters. NDVI was found to be positively correlated to PSPP, SPL, GWPS, and GWPP but negatively correlated to GPS and GPP across the three conditions

(Figure 4B–D). Reversely, PDL showed significantly negative correlations with PSPP, SPL, GWPS, and GWPP but positive correlations with GPS and GPP under C and EDS (Figure 4B,C). FLA was mainly positively correlated to yield components SPP, GPS, GPP, and TGW under C and EDS (Figure 4B,C), while strong correlations were found in FLA versus SPL (−0.51***), GWPS (−0.54***), and GWPP (−0.49***) under LDS (Figure 4D). Surprisingly, RB showed negative correlations to PSPP, SPL, GWPS, and GWPP under C (Figure 4B) while a few positive correlations were found under drought stresses such as RB versus GPS (0.45***) and GPP (0.51***) under EDS (Figure 4C).

### 3.4 | Genotypes With the Highest Stability and Adaptability of Grain Yield

The BLUP-based stability analysis on grain yield (GWPP) of all the studied genotypes showed a harmonic mean of genotypic value (HMGV) of 2.06–7.91 g, a relative performance of genotypic value (RPGV) of 0.42–1.51, and a harmonic mean of relative performance of genotypic value (HMRPGV) of 0.39–1.50 (Table S7). At the top of the list, NGB (73, 74 and 75), 2RL (37, 38 and 39), and cfAD99 (41, 42, 59 and 60) genotypes outnumbered other genotype groups, while, clearly, 1R, 1R+6R, and 5RS+4R+6R genotypes tended to fall into the bottom of the rank (Table S7).

The additive main effects and multiplicative interaction (AMMI) detected LDS as a contributing Gen-Env interaction force to reduce GWPP (Figure 5). In terms of stability and adaptability, this result matched well with the BLUP-based stability indices rank (Figure 5 and Table S7), identifying the same group of genotypes located to the mid-right (high and stable GWPP) of the biplot. Thus, 14 genotypes situated in the dashed rectangle, that is, Kr 08–101 (2RL), Kr 08–102 (2RL), Kr 08–103 (2RL), Kr 08–108 (cfAD99), Kr 08–114 (cfAD99), Kr 08–134 (5RS), Kr 08–148 (cfAD99), Kr 08–150 (cfAD99), Kr 08–153 (1RS), Kr 08–169 (TPL), Kr 08–178 (1RS), Kr 08–179 (WPL), NGB9057.3 (NGB), and NGB13.4 (NGB) were considered genotypes with stable and high grain yield across three conditions (Figure 5).

By the use of PCA on all the studied traits except GWPP for the 14 genotypes selected by AMMI analysis representing high stability and adaptability of GWPP, several genetically determined traits were found to play a substantial role in contributing to the high-yield performance (Figure 6). Under control conditions, TGW and TPP contributed to high GWPP in genotypes 41 (cfAD99), 42 (cfAD99), 61 (1RS), 70 (1RS), and 71 (WPL), while NDVI and three spike-size-related traits (SPL, GPS, and GPP) showed a high impact on GWPP in 37 (2RL), 38 (2RL), 39 (2RL), 53 (5RS), 59 (cfAD99), and 60 (cfAD99). FLA, DTH, and DTA were found to explain high GWPP in two genotypes 73 (NGB) and 74 (NGB) (Figure 6A).

Under EDS, the same combination of spike-size-related traits (SPL, GPS, and GPP) was found to contribute to high GWPP in genotypes 59 (cfAD99) and 60 (cfAD99), while in 37 (2RL), 38 (2RL), and 39 (2RL), those traits shifted to the morphological traits PDL, RB, and NDVI (Figure 6B).

Under LDS, most genotypes were concentrated in the center of the plot as a result of the strong effect from LDS. Genotypes 61 (1RS) and 70 (1RS) clearly stood out with high RB and TGW, which can partially explain their stability and adaptability of GWPP. Furthermore, TGW also showed a positive impact on GWPP in genotype 60 (cfAD99; Figure 6C).

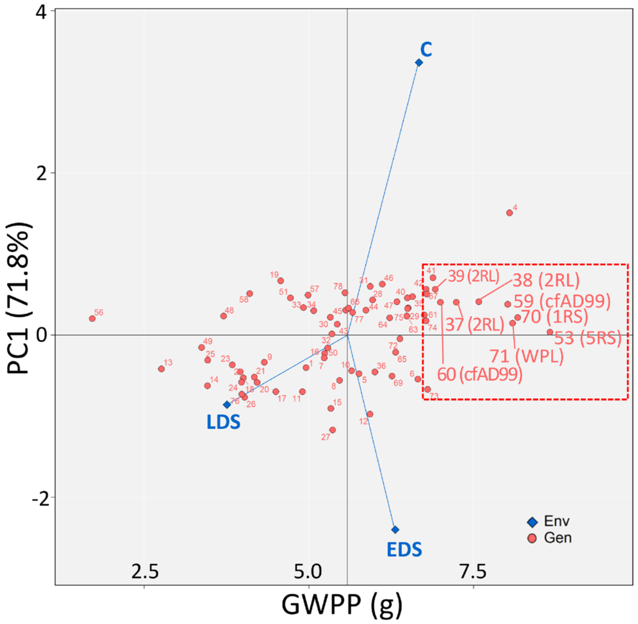

**FIGURE 5** | Additive main effects and multiplicative interaction (AMMI) biplots showing grain weight per plant (GWPP) versus the first principal component (PC1) score of 77 genotypes (Gen) and three growing conditions (Env) including control (C), early drought stress (EDS), and late drought stress (LDS). 1RS, 2RL, and 5RS: Wheat-rye introgression lines with chromosome 1RS, 2RL, and 5RS; cfAD99: Introgression lines crossed from wheat-*Leymus mollis* hybrids AD99; WPL: Wheat parental line for wheat-rye introgressions. Genotypes located closer to the horizontal axis (score 0 on PC1) showed relatively higher stability across the three growth conditions.

### 3.5 | Correlations Between the Digital Spike-Size and Yield Traits

The spike area per plant (SAPP) measured with the assistance of a machine-learning model showed significant and positive correlations with three yield traits, that is, grain weight per plant (GWPP), grains per plant (GPP), and spike length (SPL), except for the SPL under late drought stress (Figure 7). The strongest correlation was found between SAPP and GPP ($R^2 = 0.70$***; Figure 7D), and the $R^2$ of SAPP-yield-trait correlations showed a control > early drought > late drought pattern, suggesting a clear disruption by drought stresses. This trend implied that SAPP and yield traits (GWPP, GPP, and SPL) were affected differently by EDS and LDS, which was confirmed by the percent reductions (Table S8). Under EDS, the mean SAPP was slightly increased by 9.5% while GPP remained unchanged (0.9%) and GWPP showed a mild reduction of 5.7%. Under LDS, both GPP (32.4%) and GWPP (43.2%) showed a much larger reduction than SAPP (13.4%). Thus, the yield reduction by drought was mainly shown in the form of sterile spikes (reduced weight and number of grains) instead of smaller spikes. Furthermore, the relatively low correlations

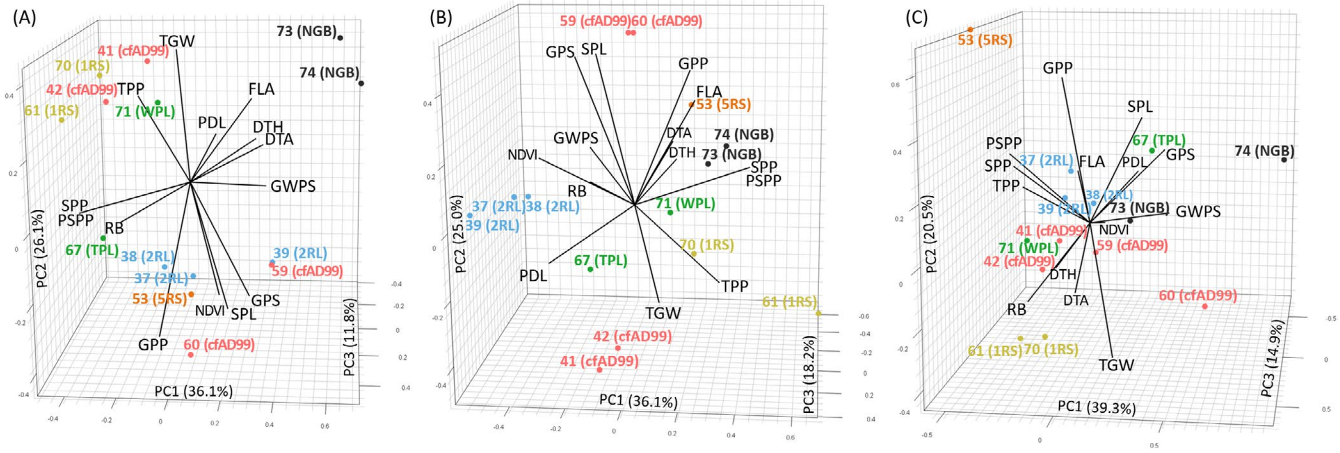

**FIGURE 6** | Principal-component analysis (PCA) using all the studied traits except grain weight per plant (GWPP) with the 14 genotypes representing high stability and adaptability of GWPP under (A) control, (B) early drought stress (EDS), and (C) late drought stress (LDS). NGB: Cultivars received from gene bank NordGen; 1RS, 2RL, 5RS: Wheat-rye introgression lines carrying rye chromosome 1RS, 2RL, and 5RS; cfAD99: Introgression lines crossed from wheat-*Leymus mollis* hybrids AD99; TPL and WPL: Triticale and wheat parental lines for wheat-rye introgressions.

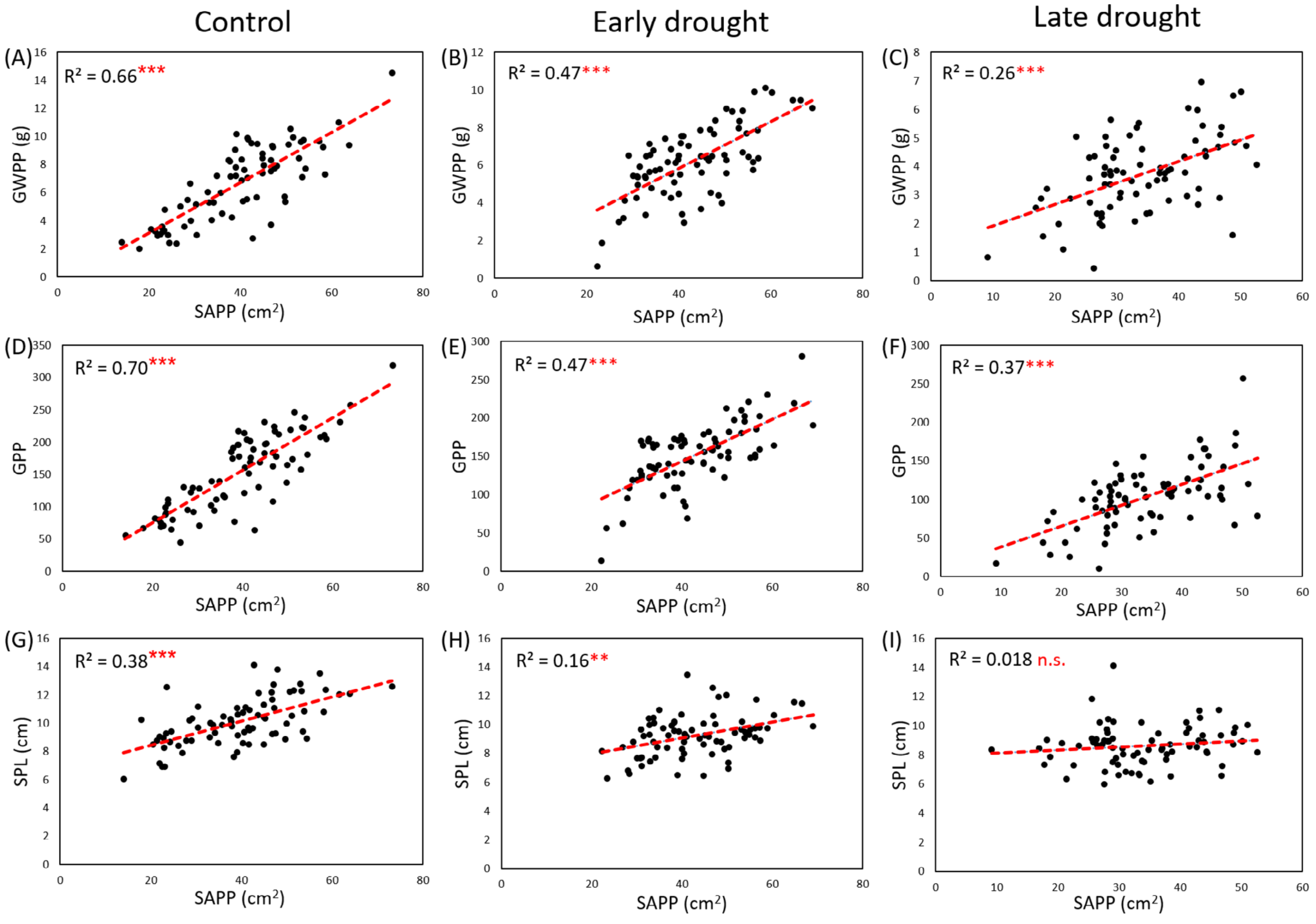

**FIGURE 7** | Linear regression of spike area per plant (SAPP) with grain weight per plant (GWPP), grains per plant (GPP) and spike length (SPL) under (A, D, G) control, (B, E, H) early drought stress, and (C, F, I) late drought stress. Data are the means of three biological replicates. ***: Sig. <0.001, **: Sig. <0.01, *: Sig. <0.05, n.s. ≥0.05.

between SAPP and SPL were mainly due to the lower variations (measured as coefficient of variation) in SPL among genotypes (C: 16.6%, EDS: 15.6% and LDS: 16.1%) as compared to other yield components (Table S8).

## 4 | Discussion

Both modern and NGB genotypes showed clearly longer early roots than old genotypes, while 1R and 1R + 6R genotypes were found to have less robust early root growth than some other introgression lines. Interestingly, the least robust old genotypes showed significantly higher root biomass at maturity than both modern and NGB genotypes. However, the large root biomass did not bring a high yield to old genotypes, which was also confirmed by the absence of positive RB-yield correlations. Instead, the mean comparison combined with stability analysis identified the best yield performance among 1RS, 2RL, and cfAD99 genotypes. Furthermore, GPS, GPP, SPL, PDL, RB, and NDVI were found to contribute to adaptability at EDS in cfAD99 and 2RL genotypes, while RB and TGW demonstrated the importance of LDS tolerance in 1RS genotypes. The strong associations between SAPP and yield components proved the promising reliability and accuracy of the methodology of extracting spike-size-related parameters from images using a machine-learning model. An increase in sterile spikes and not in spike size was found to be the main form of yield reduction induced by drought.

The significantly lower early root growth (measured as DRL), found in winter wheat in the present study, of old and 1R genotypes than that of modern and NGB genotypes was different from that of a previous study on spring wheat where old and 1R spring-wheat genotypes were found with a significantly higher DRL than modern, 2R, and 3R genotypes (Lan et al. 2022). One possible explanation for these differences between the winter and spring material might be that a spring drought is common in Nordic conditions (Roitsch et al. 2022). Thus, it is of specific importance to have a robust root system at an early stage, which will contribute with opportunities for spring-sown crops to access water from deep soil. Previous studies have indicated a robust root system improves the survival rate of young seedlings when drought occurs (Regan et al. 1992). Differently from the spring wheat, winter wheat is sown in the autumn, and then enters and remains at the vernalization stage throughout the entire winter where soil water content is usually sufficient (decent precipitation and low evaporation rate) in Nordic conditions and plant growth remains at a minimum rate (low transpiration, respiration, and photosynthesis; Li et al. 2013). Thus, the advantages of a vigorous early root system are naturally less demanded by winter wheat, especially not for old cultivars, landraces, and primitives which are well adapted to local natural conditions and which have received less genetic modification than modern winter wheat. Combined with our previous finding (Lan et al. 2022), the early vigor results of the present study pointed out that breeding targets of early traits need to be adjusted according to the actual demand by spring and winter wheat.

Different from early vigor, old Swedish cultivars, along with 1R and 1RS + 2RL genotypes, demonstrated in this study a more robust root system at maturity. This finding corresponded well with several previous observations that old wheat generally showed a larger root system than modern wheat (Bektas et al. 2016; Lan et al. 2022; Zhang et al. 2020), which was attributed to the modern yield-oriented breeding programs that have altered the above- and under-ground biomass allocation of wheat plants (Bektas and Waines 2018). In wheat-rye introgression lines, the translocation of 1RS has been reported to significantly increase the root dry weight of winter wheat (Hoffmann 2008). In addition to the root traits, successful introgression of 1RS has also been found to improve the final yield (Kim et al. 2004; Ren et al. 2018) and resistance to a wide spectrum of wheat diseases, for example, stem rust (Anugrahwati et al. 2008), stripe rust (Szakács et al. 2020), leaf rust (Hsam et al. 2000) and powdery mildew (Ren et al. 2018). However, wheat lines carrying 1RS have been widely reported with the issue of quality defects such as sticky and weak dough, which was attributed to the replacement of gluten-strength-related genes on 1B by secalin genes on 1R that do not contribute to gluten strength and overall make gluten weak (Dhaliwal et al. 1987; Graybosch et al. 1993). The major quality defect-related genes are likely to be situated on the 1RS arm (Li et al. 2016), which in a previous study on spring wheat resulted in low polymerization (measured as %UPP with SE-HPLC) observed in 1RS genotypes independent of the three (C, EDS, and LDS) conditions utilized (Lan et al. 2023).

Similarly to the spring wheat reported by Lan et al. (2022), the root biomass at maturity in the present study showed an overall negative correlation with yield traits. Moreover, neither the robust early root growth nor the final root biomass was found to translate into high yields in this study, which contradicted the positive root–yield relationship reported by other studies (Ehdaie et al. 2012; Heřmanská et al. 2015). However, these studies were carried out in field conditions that allowed roots to expand freely, while in the present study, the root movements were limited in a 2.5 L pot. The wheat plants showing positive relationships between root and shoot biomass in indoor conditions were mainly grown in customized containers with a vertical elongation for root growth, for example, 1.5 m PVC tubes (Bektas et al. 2016) and 1.6 m columns (Friedli et al. 2019). Thus, space limitation and the lack of a positive effect by reaching additional water and nutrients with a larger root system are the main reasons causing the lack of positive root–yield correlations here.

Increasing the grain yield and stabilizing it across varying conditions are the ultimate breeding goals in response to the exacerbating climate change (Rajaram et al. 1997). Our BLUP-based stability analysis detected NGB, 2RL, and cfAD99 as the three genotype groups with the best resilience (represented by HMGV, RPGV, and HMRPGV) across C, EDS, and LDS. Furthermore, AMMI results suggested Kr 08–101 (2RL), Kr 08–102 (2RL), Kr 08–103 (2RL), Kr 08–108 (cfAD99), Kr 08–114 (cfAD99), Kr 08–134 (5RS), Kr 08–148 (cfAD99), Kr 08–150 (cfAD99), Kr 08–153 (1RS), Kr 08–169 (TPL), Kr 08–178 (1RS), Kr 08–179 (WPL), NGB9057.3 (NGB), and NGB13.4 (NGB) as the 14 genotypes with a high and stable grain yield across three conditions. Clearly, genotypes with the genetic background of the wheat-*Leymus mollis* accession AD99 stood out with promising adaptability to drought. *Leymus mollis*, the wild relative of common wheat, is known for its strong resilience to harsh environments, for example, drought stress (Habora et al. 2012) and fungal diseases (Li et al. 2015). The four cfAD99 genotypes highlighted by our results implied the potential presence of drought-related genes in *the Leymus mollis* genome, and therefore, further studies aiming for the identification and successful transfers of those genes into wheat are of great significance. Chromosome 2RL has been largely exploited for resistance to abiotic and biotic stress such as powdery mildew (An et al. 2022), stem rust (Rahmatov et al. 2016), and hessian fly (Cainong et al. 2010; Friebe et al. 1990), while it, however, has not been reported to carry genes that contribute to drought tolerance. An increased grain number with the presence of 2RL was recorded in a study, which corresponded well with our GPP data, but no positive effect of 2RL on grain yield was found there (Ehdaie et al. 2003). Thus, the expression of genes on 2RL is likely dependent on the interactions with the local genes of the recipient, and more studies are needed to search for drought/yield-related genes on 2RL.

Interestingly, the high grain yield of 2RL genotypes can be decomposed into yield components GPS, GPP, and SPL under control, while the traits accounting for tolerance to EDS in 2RL genotypes were found morphology- and physiology-wise, for example, PDL, RB, and NDVI. The early drought that takes place during vegetative growth is known to mainly affect morphological traits, and then, as a result of the morphological suppressions, final yield can be influenced (Hafid et al. 1998; Lan et al. 2022). Thus, the impact of early drought on yield is usually milder than that of late drought, which harms plants directly at the most critical reproductive stages (Van Ginkel et al. 1998). The plants demonstrating a strong morphologic-growth-maintaining ability under early drought are likely to restore normal growth soon after the stress, which minimizes the impact on the final yield. Therefore, 37 (2RL), 38 (2RL), and 39 (2RL) are considered specifically tolerant to early drought here, and chromosome 2RL is likely to carry genes contributing to sustaining vegetative growth under early drought. TGW and TPP were found to contribute to the high grain yield of 61 (1RS) and 70 (1RS) genotypes under control, while the tolerance to late drought of these two genotypes was positively correlated to TGW and RB. Thus, the large grain size might play a role in maintaining grain yield under late drought, which corresponds well to our previous study (Lan et al. 2022). Chromosome 1RS has been reported to confer tolerance to drought at the mid-flowering stage in winter wheat by increasing root dry weight, harvest index, and water use efficiency (Hoffmann 2008). Clearly, the four cfAD99 genotypes selected by AMMI can be divided into two groups because their grain yield was explained by two sets of traits that negatively correlated to each other, that is, TGW and TPP contributing to the high yield of genotypes 41 and 42 under control and EDS, and GPS, GPP, and SPL contributing to the high yield of genotypes 59 and 60 under control and EDS. Genotypes 41 and 42 share the same breeding path (AD99 × Goerzen) × vete, while genotypes 59 and 60 are both bred through (AD99 × Kraka höstvete) × Kraka höstvete (Table S1). Thus, despite the fact that the four genotypes with high and stable grain yield share the parent AD99, the difference between Goerzen and Kraka höstvete in their pedigree resulted in distinct components that constitute the resilience of grain yield.

The strong positive correlations between SAPP and the other three yield traits proved the accuracy of the image-based spike size trait measured. In addition to the accuracy, this digital phenotyping assisted by a machine-learning model also showed advances in efficiency and consistency. After the model is trained, all the images can be batch processed in one go for spike detection, and all the operations from spike segmentation to the final spike area measurement can be automated by a pre-written script. Thus, this methodology avoided the labor-intensive and time-consuming features of traditional measurements. Furthermore, our phenotyping technique also eliminated the subjectivity that is widely present in large-scale manual measurements because those types of measurements usually require several people working in parallel, but a slight difference in measuring standards could cause error-prone results. Thus, the image-based phenotyping assisted by a machine-learning model used in the present study is a promising technique with high accuracy, efficiency, and consistency. The trainable Weka segmentation has been widely utilized in different scientific areas, for example, emulsion droplet size determination (Salum et al. 2022), tissue segmentation (Polan et al. 2016), panoramic radiographs (Kanuri et al. 2022) and road extraction from unmanned aerial vehicle images (Abdollahi et al. 2019). In combination with the much higher percent reductions of GWPP (43.2%) and GPP (32.4%) than SAPP (13.4%) under LDS, the weakened correlations between SAPP and yield traits suggested that instead of smaller spikes, sterile spikes (reduced weight and number of grains) are the major cause of yield reduction under late drought, which is in agreement with other studies (Dong et al. 2017; Ji et al. 2010).

## 5 | Conclusions

The varying climate is increasing the uncertainties of food security, constituted by quality, stability, and productivity. As a result of global warming, drought has become the major limiting factor to crop yield. To increase the resilience of wheat, relevant genes need to be searched among old, primitive, landrace wheat lines as well as its wild relative rye. Early root vigor is considered a useful characteristic to sustain the growth of spring wheat under drought. However, the low adaptability of early root growth observed in old Swedish cultivars here suggests its low importance in winter wheat due to the fact that these obtain vernalization during the winter when the seedlings have a low demand for water and the water deficit is not likely to happen during Nordic winter. The rye chromosomes 2RL and 1RS as well as chromosomes from *Leymus mollis* accession AD99 may contain genes contributing to drought tolerance in winter wheat. Thus, introgression lines with those chromosomes should be widely tested and utilized in breeding programs to develop resilient germplasms. Under early drought, peduncle length, root biomass, and NDVI are likely to contribute to stabilizing grain yield of 2RL genotypes, while grains per spike, grains per plant, and spike length are dominating to stabilize grain yield of cfAD99 genotypes. Root biomass and 1000-grain weight contribute to high and stable grain yield of 1RS genotypes under late drought. Here, a machine-learning model is found to be a powerful approach to quantifying spike area from images. The digital spike area combined with yield reduction proves that the drought-induced grain yield loss is mainly caused by reduced sterility (lowered grain number and weight) instead of changes in spike size. Therefore, breeders should give more priority to winter breeding lines with vigorous pollen fertility under drought conditions.

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### Author Contributions

**Yuzhou Lan:** conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; roles/writing – original draft; writing – review and editing. **Aakash Chawade:** conceptualization; methodology; supervision; roles/writing – review and editing. **Ramune Kuktaite:** conceptualization; methodology; supervision; roles/writing – review and editing. **Eva**

**Johansson:** conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review and editing.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that supports the findings of this study are available in the Table S3.

## References

Abdollahi, A., B. Pradhan, and N. Shukla. 2019. "Extraction of Road Features From UAV Images Using a Novel Level Set Segmentation Approach." *International Journal of Urban Sciences* 23, no. 3: 391–405.

Afzal, F., S. K. Chaudhari, A. Gul, et al. 2015. "Bread Wheat (*Triticum aestivum* L.) Under Biotic and Abiotic Stresses: An Overview." In *Crop Production and Global Environmental Issues*, edited by K. Hakeem, 293–317. Springer.

Alvarez-Morezuelas, A., L. Barandalla, E. Ritter, M. Lacuesta, and J. I. Ruiz de Galarreta. 2022. "Physiological Response and Yield Components Under Greenhouse Drought Stress Conditions in Potato." *Journal of Plant Physiology* 278: 153790. https://doi.org/10.1016/j.jplph.2022.153790.

An, D., G. Han, J. Wang, et al. 2022. "Cytological and Genetic Analyses of a Wheat-Rye 2RL Ditelosomic Addition Line With Adult Plant Resistance to Powdery Mildew." *Crop Journal* 10, no. 3: 911–916.

Anugrahwati, D. R., K. W. Shepherd, D. C. Verlin, et al. 2008. "Isolation of Wheat–Rye 1RS Recombinants That Break the Linkage Between the Stem Rust Resistance Gene SrR and Secalin." *Genome* 51, no. 5: 341–349.

Armonienė, R., F. Odilbekov, V. Vivekanand, and A. Chawade. 2018. "Affordable Imaging Lab for Noninvasive Analysis of Biomass and Early Vigour in Cereal Crops." *BioMed Research International* 2018: 5713158. https://doi.org/10.1155/2018/5713158.

Bektas, H., C. E. Hohn, and J. G. Waines. 2016. "Root and Shoot Traits of Bread Wheat (*Triticum aestivum* L.) Landraces and Cultivars." *Euphytica* 212, no. 2: 297–311. https://doi.org/10.1007/s10681-016-1770-7.

Bektas, H., and J. G. Waines. 2018. "Root and Shoot Traits in Parental, Early and Late Generation Green Revolution Wheats (Triticum spp.) Under Glasshouse Conditions." *Genetic Resources and Crop Evolution* 65, no. 7: 2003–2012. https://doi.org/10.1007/s10722-018-0673-y.

Cainong, J., L. Zavatsky, M. Chen, et al. 2010. "Wheat-Rye T2BS· 2BL-2RL Recombinants With Resistance to Hessian Fly (H21)." *Crop Science* 50, no. 3: 920–925.

Cook, B. I., J. S. Mankin, and K. J. Anchukaitis. 2018. "Climate Change and Drought: From Past to Future." *Current Climate Change Reports* 4, no. 2: 164–179. https://doi.org/10.1007/s40641-018-0093-2.

Crofts, H. J. 1989. "On Defining a Winter Wheat." *Euphytica* 44, no. 3: 225–234. https://doi.org/10.1007/BF00037529.

Dai, A., T. Zhao, and J. Chen. 2018. "Climate Change and Drought: A Precipitation and Evaporation Perspective." *Current Climate Change Reports* 4, no. 3: 301–312. https://doi.org/10.1007/s40641-018-0101-6.

Dhaliwal, A., D. Mares, and D. Marshall. 1987. "Effect of 1B/1R Chromosome Translocation on Melling and Quality Characteristics of Bread Wheats." *Cereal Chemistry* 64, no. 2: 72–76.

Dickin, E., and D. Wright. 2008. "The Effects of Winter Waterlogging and Summer Drought on the Growth and Yield of Winter Wheat (*Triticum aestivum* L.)." *European Journal of Agronomy* 28, no. 3: 234–244.

Dong, B., X. Zheng, H. Liu, et al. 2017. "Effects of Drought Stress on Pollen Sterility, Grain Yield, Abscisic Acid and Protective Enzymes in Two Winter Wheat Cultivars." *Frontiers in Plant Science* 8: 1008.

Ehdaie, B., A. P. Layne, and J. G. Waines. 2012. "Root System Plasticity to Drought Influences Grain Yield in Bread Wheat." *Euphytica* 186, no. 1: 219–232.

Ehdaie, B., R. Whitkus, and J. Waines. 2003. "Root Biomass, Water-Use Efficiency, and Performance of Wheat–Rye Translocations of Chromosomes 1 and 2 in Spring Bread Wheat 'Pavon'." *Crop Science* 43, no. 2: 710–717.

Entz, M., and D. Fowler. 1991. "Agronomic Performance of Winter Versus Spring Wheat." *Agronomy Journal* 83, no. 3: 527–532.

Fang, Y., Y. Du, J. Wang, et al. 2017. "Moderate Drought Stress Affected Root Growth and Grain Yield in Old, Modern and Newly Released Cultivars of Winter Wheat." *Frontiers in Plant Science* 8: 672. https://doi.org/10.3389/fpls.2017.00672.

Fang, Y., L. Liu, B.-C. Xu, and F.-M. Li. 2011. "The Relationship Between Competitive Ability and Yield Stability in an Old and a Modern Winter Wheat Cultivar." *Plant and Soil* 347, no. 1: 7–23. https://doi.org/10.1007/s11104-011-0780-4.

Foulkes, M., R. Sylvester-Bradley, R. Weightman, and J. Snape. 2007. "Identifying Physiological Traits Associated With Improved Drought Resistance in Winter Wheat." *Field Crops Research* 103, no. 1: 11–24.

Friebe, B., J. Hatchett, R. Sears, and B. Gill. 1990. "Transfer of Hessian Fly Resistance From 'Chaupon' Rye to Hexaploid Wheat via a 2BS/2RL Wheat-Rye Chromosome Translocation." *Theoretical and Applied Genetics* 79: 385–389.

Friedli, C. N., S. Abiven, D. Fossati, and A. Hund. 2019. "Modern Wheat Semi-Dwarfs Root Deep on Demand: Response of Rooting Depth to Drought in a Set of Swiss Era Wheats Covering 100 Years of Breeding." *Euphytica* 215, no. 4: 85.

Fufa, H., P. S. Baenziger, B. Beecher, R. A. Graybosch, K. M. Eskridge, and L. A. Nelson. 2005. "Genetic Improvement Trends in Agronomic Performances and End-Use Quality Characteristics Among Hard Red Winter Wheat Cultivars in Nebraska." *Euphytica* 144, no. 1: 187–198.

Graybosch, R., C. Peterson, L. Hansen, D. Worrall, D. Shelton, and A. Lukaszewski. 1993. "Comparative Flour Quality and Protein Characteristics of 1BL/1RS and 1AL/1RS Wheat-Rye Translocation Lines." *Journal of Cereal Science* 17, no. 2: 95–106.

Habora, M. E. E., A. E. Eltayeb, H. Tsujimoto, and K. Tanaka. 2012. "Identification of Osmotic Stress-Responsive Genes From *Leymus mollis*, a Wild Relative of Wheat (*Triticum aestivum* L.)." *Breeding Science* 62, no. 1: 78–86.

Hafid, R. E., D. H. Smith, M. Karrou, and K. Samir. 1998. "Morphological Attributes Associated With Early-Season Drought Tolerance in Spring Durum Wheat in a Mediterranean Environment." *Euphytica* 101: 273–282.

Heřmanská, A., T. Středa, and O. Chloupek. 2015. "Improved Wheat Grain Yield by a New Method of Root Selection." *Agronomy for Sustainable Development* 35: 195–202.

Hoffmann, B. 2008. "Alteration of Drought Tolerance of Winter Wheat Caused by Translocation of Rye Chromosome Segment 1RS." *Cereal Research Communications* 36, no. 2: 269–278.

Hsam, S., V. Mohler, L. Hartl, G. Wenzel, and F. Zeller. 2000. "Mapping of Powdery Mildew and Leaf Rust Resistance Genes on the Wheat-Rye Translocated Chromosome T1BL· 1RS Using Molecular and Biochemical Markers." *Plant Breeding* 119, no. 1: 87–89.

Ji, X., B. Shiran, J. Wan, et al. 2010. "Importance of Pre-Anthesis Anther Sink Strength for Maintenance of Grain Number During Reproductive Stage Water Stress in Wheat." *Plant, Cell and Environment* 33, no. 6: 926–942.

Johansson, E., R. Kuktaite, M. Labuschagne, et al. 2023. "Adaptation to Abiotic Stress Factors and Their Effects on Cereal and Pseudocereal Grain Quality." In *Developing, Sustainable and Health Promoting Cereals and Pseudocereals*, 339–358. Elsevier.

Kanuri, N., A. Z. Abdelkarim, and S. A. Rathore. 2022. "Trainable WEKA (Waikato Environment for Knowledge Analysis) Segmentation Tool: Machine-Learning-Enabled Segmentation on Features of Panoramic Radiographs." *Cureus* 14, no. 1: e21777.

Kim, W., J. W. Johnson, P. S. Baenziger, A. J. Lukaszewski, and C. S. Gaines. 2004. "Agronomic Effect of Wheat-Rye Translocation Carrying Rye Chromatin (1R) From Different Sources." *Crop Science* 44, no. 4: 1254–1258. https://doi.org/10.2135/cropsci2004.1254.

Kumar, D., S. Kushwaha, C. Delvento, et al. 2020. "Affordable Phenotyping of Winter Wheat Under Field and Controlled Conditions for Drought Tolerance." *Agronomy* 10, no. 6: 882.

Lama, S., F. Leiva, P. Vallenback, A. Chawade, and R. Kuktaite. 2023. "Impacts of Heat, Drought, and Combined Heat–Drought Stress on Yield, Phenotypic Traits, and Gluten Protein Traits: Capturing Stability of Spring Wheat in Excessive Environments." *Frontiers in Plant Science* 14: 1179701. https://doi.org/10.3389/fpls.2023.1179701.

Lan, Y., A. Chawade, R. Kuktaite, and E. Johansson. 2022. "Climate Change Impact on Wheat Performance—Effects on Vigour, Plant Traits and Yield From Early and Late Drought Stress in Diverse Lines." *International Journal of Molecular Sciences* 23, no. 6: 3333. https://doi.org/10.3390/ijms23063333.

Lan, Y., R. Kuktaite, A. Chawade, and E. Johansson. 2023. "Diverse Wheat Lines to Mitigate the Effect of Drought on End-Use Quality." *Frontiers in Food Science and Technology* 3: 1163412. https://doi.org/10.3389/frfst.2023.1163412.

Langridge, P., and M. Reynolds. 2021. "Breeding for Drought and Heat Tolerance in Wheat." *Theoretical and Applied Genetics* 134: 1753–1769.

Li, G., M. Yu, T. Fang, S. Cao, B. F. Carver, and L. Yan. 2013. "Vernalization Requirement Duration in Winter Wheat Is Controlled by TaVRN-A1 at the Protein Level." *Plant Journal* 76, no. 5: 742–753.

Li, H., R. Fan, S. Fu, et al. 2015. "Development of *Triticum aestivum-Leymus mollis* Translocation Lines and Identification of Resistance to Stripe Rust." *Journal of Genetics and Genomics* 42, no. 3: 129–132.

Li, Z., T. Ren, B. Yan, F. Tan, M. Yang, and Z. Ren. 2016. "A Mutant With Expression Deletion of Gene Sec-1 in a 1RS.1BL Line and Its Effect on Production Quality of Wheat." *PLoS One* 11, no. 1: e0146943. https://doi.org/10.1371/journal.pone.0146943.

Mohammadi, R., E. Farshadfar, M. Aghaee-Sarbarzeh, and J. Sutka. 2003. "Locating QTLs Controlling Drought Tolerance Criteria in Rye Using Disomic Addition Lines." *Cereal Research Communications* 31: 257–264.

Polan, D. F., S. L. Brady, and R. A. Kaufman. 2016. "Tissue Segmentation of Computed Tomography Images Using a Random Forest Algorithm: A Feasibility Study." *Physics in Medicine and Biology* 61, no. 17: 6553–6569.

Pound, M. P., A. P. French, J. A. Atkinson, D. M. Wells, M. J. Bennett, and T. Pridmore. 2013. "RootNav: Navigating Images of Complex Root Architectures." *Plant Physiology* 162, no. 4: 1802–1814.

Rahmatov, M., M. N. Rouse, J. Nirmala, et al. 2016. "A New 2DS· 2RL Robertsonian Translocation Transfers Stem Rust Resistance Gene Sr59 Into Wheat." *Theoretical and Applied Genetics* 129: 1383–1392.

Rajaram, S., H.-J. Braun, and M. van Ginkel. 1997. "CIMMYT'S Approach to Breed for Drought Tolerance." In *Adaptation in Plant Breeding*, 161–167. Springer.

Regan, K., K. Siddique, N. Turner, and B. Whan. 1992. "Potential for Increasing Early Vigour and Total Biomass in Spring Wheat. II. Characteristics Associated With Early Vigour." *Australian Journal of Agricultural Research* 43, no. 3: 541–553.

Ren, T., Z. Ren, M. Yang, et al. 2018. "Novel Source of 1RS From Baili Rye Conferred High Resistance to Diseases and Enhanced Yield Traits to Common Wheat." *Molecular Breeding* 38, no. 8: 1–9.

Roitsch, T., K. Himanen, A. Chawade, L. Jaakola, A. Nehe, and E. Alexandersson. 2022. "Functional Phenomics for Improved Climate Resilience in Nordic Agriculture." *Journal of Experimental Botany* 73, no. 15: 5111–5127. https://doi.org/10.1093/jxb/erac246.

RStudio Team. 2015. *RStudio: Integrated Development for R*, 700. RStudio Inc.

Salum, P., O. Güven, L. Y. Aydemir, and Z. Erbay. 2022. "Microscopy-Assisted Digital Image Analysis With Trainable Weka Segmentation (TWS) for Emulsion Droplet Size Determination." *Coatings* 12, no. 3: 364.

Shiferaw, B., M. Smale, H.-J. Braun, E. Duveiller, M. Reynolds, and G. Muricho. 2013. "Crops That Feed the World 10. Past Successes and Future Challenges to the Role Played by Wheat in Global Food Security." *Food Security* 5, no. 3: 291–317.

Szakács, É., K. Szőke-Pázsi, B. Kalapos, et al. 2020. "1RS Arm of *Secale cereanum* 'Kriszta' Confers Resistance to Stripe Rust, Improved Yield Components and High Arabinoxylan Content in Wheat." *Scientific Reports* 10, no. 1: 1792.

Van Ginkel, M., D. Calhoun, G. Gebeyehu, et al. 1998. "Plant Traits Related to Yield of Wheat in Early, Late, or Continuous Drought Conditions." *Euphytica* 100: 109–121.

Wang, X., L. Wang, and Z. Shangguan. 2016. "Leaf Gas Exchange and Fluorescence of Two Winter Wheat Varieties in Response to Drought Stress and Nitrogen Supply." *PLoS One* 11, no. 11: e0165733. https://doi.org/10.1371/journal.pone.0165733.

Zhang, L., Y.-L. Du, and X. G. Li. 2020. "Modern Wheat Cultivars Have Greater Root Nitrogen Uptake Efficiency Than Old Cultivars." *Journal of Plant Nutrition and Soil Science* 183, no. 2: 192–199.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **FIGURE S1:** fes370116-sup-0001-Figure S1.tiff. **Table S1:** fes370116-sup-0002-TableS1.xlsx. **Table S2:** fes370116-sup-0003-TableS2.xlsx. **Table S3:** fes370116-sup-0004-TableS3.xlsx. **Table S4:** fes370116-sup-0005-TableS4.xlsx. **Table S5:** fes370116-sup-0006-TableS5.xlsx. **Table S6:** fes370116-sup-0007-TableS6.xlsx. **Table S7:** fes370116-sup-0008-TableS7.xlsx. **Table S8:** fes370116-sup-0009-TableS8.xlsx.

## Appendix A

```
run(“8-bit”);

setOption(“BlackBackground”, false);

run(“Convert to Mask”);

run(“Create Selection”);

run(“Measure”);
```