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ORIGINAL ARTICLE

Dissecting lentil crop growth in contrasting environments using digital imaging and genome-wide association studies

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Abstract

The development of high-throughput phenotyping platforms to capture time-series data on large, diverse populations holds promise for crop researchers and breeders investigating growth-related traits. We used imagery from unoccupied aerial vehicles (UAVs) with red/green/blue (RGB) and multispectral cameras flown over multiple site-years in Saskatchewan, Canada, and Metaponto, Italy, to gather data for crop height, area, and volume in a lentil diversity panel (324 genotypes). The temporal nature of the UAV image-derived data enabled the modeling of growth curves for volume, height, and area, something that would be impractical under traditional phenotyping procedures in such a large population grown in multiple environments. A principal component analysis and hierarchical clustering revealed differential growth patterns across contrasting environments, with large variations in temperature and photoperiod, within our lentil diversity panel. Combining this analysis with genome-wide genotyping data, we identified markers, from an exome capture array (267,845 single nucleotide polymorphisms), associated with crop growth that could be used for marker-assisted selection. Our study demonstrates the potential for UAV-based imaging to obtain large-scale time-series data across multiple environments to model growth curves and investigate genotype-by-environment interactions. In addition, we can now use phenotypic traits that were once impractical to collect and derive novel phenotypes to improve our understanding of crop growth and the genetics underlying adaptation in lentil, approaches that will be useful for both researchers and breeders.

Plain Language Summary

We used drones to image lentil plants in Canada and Italy throughout multiple growing seasons. These images helped us measure the height, area, and volume of

Abbreviations: DTF, days from sowing to flower; G × E, genotype by environment; GWAS, genome-wide association study; LDP, lentil diversity panel; PCA, principal component analysis; QTL, quantitative trait loci; UAV, unoccupied aerial vehicle.

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individual plots over time. This method is much more efficient than traditional ways of gathering growth data from large numbers of plants in different environments. By analyzing the data, we found different growth patterns in the growth pattern of lentils depending on where they originated from and where they were grown. We combined this information with genetic data to find specific markers that could help in breeding improved lentils.

1 | INTRODUCTION

Crop growth and plant architecture are agronomic and economically important traits, which are both complex in nature and strongly influenced by the environment. As such, our understanding of the underlying genetic control is often limited by the difficulty of obtaining high-quality, temporal data, relevant to the field conditions they will be cultivated in. For researchers working with large-scale, multi-environment field trials involving diverse populations, traditional phenotyping techniques have constraints, which can be overcome with high-throughput phenotyping platforms. For example, biomass measurements usually require destructive sampling, making it difficult to obtain continuous, temporal data from the same plot. In addition, some traits, such as plot width or area, can be too subjective to provide reliable data on a large scale. Unoccupied aerial vehicles (UAVs) equipped with cameras and sensors have the potential to provide less subjective, higher quality, time-series data for large populations, such as those used in breeding programs (Bhandari et al., 2023). This provides the opportunity to accurately model crop growth and estimate crop growth rates in the field and across environments.

Plant architecture is important, but manual phenotyping methods for classification are lacking in some crops. Barulina (1930) first characterized variability in lentil plant architecture into three growth habits: “erect,” “prostrate,” and “intermediate.” This was updated in 2010 with the addition of “semi-prostrate,” “upright,” and “other” (Bioversity International, International Center for Agricultural Research in the Dry Areas & National Bureau of Plant Genetic Resources, 2010), however, classification into these six groups is confusing, and researchers have noted that even two or three categories can be difficult for characterization (Sastry & Jeberson, 2021). Further complicating this is the effect of genotype by environment (G × E) interactions on plant growth. Using UAV-based imaging to estimate biomass via a plot volume estimate and canopy height has garnered considerable attention in various crop species, including maize (Adak, Conrad, et al., 2021; Adak, Murray, et al., 2021; Anderson et al., 2020; Wang et al., 2021), soybean (Li et al., 2022; Roth et al., 2022), wheat (Holman et al., 2016), rice (Taniguchi et al., 2022), and pea (Tefera et al., 2022). Previous work with some of the genotypes used in this study has been done to ground truth the data derived from UAV images with manual measurements of biomass, demonstrating the potential of this approach in lentil (Nielsen et al., 2022). Lentil plant architecture could be represented by a combination of plant height and biomass, both of which influence other traits such as yield (Lake & Sadras, 2021; Silim et al., 1993; Whitehead et al., 2000), lodging (Hanlan et al., 2006) and machine harvestability (Erskine & Goodrich, 1991; Jawad et al., 2019). The ability to estimate canopy height, plot volume, and plot area over time using UAV-based imagery provides potential to improve our characterization of lentil plant architecture throughout the growing season.

Little research has been done to investigate the genetics underlying crop growth in lentil. To our knowledge there are only a few studies identifying quantitative trait loci (QTL) associated with plant architecture (Fratini et al., 2007; Kumar et al., 2018; Tullu et al., 2008), and those have not been associated with a reference genome. Recent improvements in lentil genomic resources (Ramsay et al., 2021) have improved our ability to understand underlying factors in plant growth and production. Using genotypic data from a lentil SNP assay (e.g., Ogutcen et al., 2018), researchers can now perform more complex investigations into the genetics contributing to differences in traits across diverse germplasm. Successfully leveraging these same genomic resources, we investigated the genetics associated with variability in phenology in a lentil diversity panel (LDP) (Neupane et al., 2022). The same multi-site-year trial was also imaged via UAV throughout each growing season at 4 of the 18 site-years. The rationale behind this study was that we should be able to use existing genomic resources in combination with available high-throughput data collected using UAV-based imagery to discover new QTL related to lentil growth and plant architecture.

Our study objectives were to (a) investigate the feasibility of applying UAV-acquired imagery to estimate various growth-related parameters and model crop growth; (b) evaluate the diversity in growth-related traits among diverse lentil accessions and across environments; (c) dissect the genetics of crop growth in lentil under contrasting growing environments; and (d) explore the potential of derived phenotypes to aid in the analysis of lentil growth. The goal was to improve our understanding of lentil adaptation across diverse environments and identify QTL associated with lentil plant growth and development.

2 | MATERIALS AND METHODS

2.1 | Field experiments and manual phenotyping

An LDP consisting of 324 lentil genotypes gathered from the gene banks of the International Center for Agricultural Research in the Dry Areas (ICARDA), United States Department of Agriculture, and Plant Gene Resources of Canada, as well as cultivars developed at the Crop Development Centre, University of Saskatchewan, Canada, was evaluated over 4 site-years in Sutherland, Canada, 2017 (Su17); Sutherland, Canada, 2018 (Su18); Rosthern, Canada, 2017 (Ro17); and Metaponto, Italy, 2017 (It17). For further details see Wright et al. (2021). These locations were selected as representative of spring-sown temperate (Ro17, Su17, and Su18) and fall-sown Mediterranean (It17) macro-environments for cultivated lentil, as illustrated by the difference in phenology (Figure 1A) and experience of contrasting photoperiods and temperatures during the lentil growing season (Figure 1B,C). The field trials were arranged in a randomized lattice square (18 × 18) experimental design with three replications in each site year.

Manually collected measurements of plot height were recorded using a meter scale and taken at swollen pod stage (when 10% of plants per plot had one swollen pod). Aboveground plot biomass was measured using a scale after each plot had been harvested and dried. Details for additional manually collected traits, such as days from sowing to flower (DTF), can be found in Wright et al. (2021).

2.2 | Image acquisition and processing

Two Draganfly quadcopter UAVs (Draganfly Innovations Inc.), X4-P and Commander, were used interchangeably for image acquisition in Canada. Images were collected using consumer-grade cameras: 24.3 MP Sony a5100 or 20.1 MP Sony QX1 at Sutherland, Canada 2017, and Rosthern, Canada, 2017. A MicaSense RedEdge 3 multispectral camera (MicaSense Inc.) was used for the image acquisition at Sutherland 2018. The UAV had been programmed to fly the trial autonomously at 15 or 20 m above ground level at a ground speed of 1.7 m per second. This allowed low ground sample distance to provide high-resolution imagery. The images were captured from the nadir view while maintaining 70% front and 70% side image overlap throughout the mission.

A DJI Inspire 1 Pro UAV (DJI) was used for image acquisition at Metaponto, Italy. Images were collected using the 16 MP DJI Zenmuse X5 camera. The UAV was programmed to fly in autonomous mode at an altitude of 15 or 20 m above ground level, with an optimized ground speed to ensure accurate coverage. Images were acquired with a nadir orientation. The mission followed an image overlap strategy with a 90% frontal overlap and a 70% lateral sidelap, ensuring dense coverage and correct photogrammetric reconstruction.

Core Ideas

- Unoccupied aerial vehicles–based imaging provides a non-destructive method for constructing lentil crop growth models.
- Growth models varied considerably across diverse lentil genotypes.
- Growth models from one environment did not predict what happens in another environment.
- Regions of the genome controlling different components of the growth models were identified.

After imaging the field trials, individual images were stitched together, and orthomosaics of each flight date were generated using Pix4Dmapper (https://www.pix4d.com/product/pix4dmapper-photogrammetry-software/). Further image analyses were carried out using PlotVision (https://plotvision.usask.ca/) following the default methods to obtain vegetative indices (Blue Normalized Difference Vegetative Index or Excess Green) from which plot height, plot area, and plot volume were derived (https://plotvision-docs.readthedocs.io/en/latest/index.html). This image preprocessing and data extraction workflow is similar to the previous studies reported by Duddu et al. (2019) and Nielsen et al. (2022) who also validated the use of UAV imagery for measuring growth-related traits in lentils.

2.3 | Data wrangling, visualization, and modeling of growth curves

Data wrangling and visualization were done in R (R Core Team, 2019) using the packages “ggbeeswarm” (Clarke & Sherrill-Mix, 2017), “ggpubr” (Kassambara, 2025), and “tidyverse” (Wickham et al., 2019). Modeling of growth curves was performed using the ‘growthcurver’ package (Sprouffske & Wagner, 2016) along with additional user-calculated traits as described in Figure 2. G × E analysis was done with “lme4” using linear mixed models (Bates et al., 2015). Principal component analysis (PCA) and hierarchical *k*-means clustering were performed using the “FactoMineR” R package (Lê et al., 2008). The source code for all data analyses is available at: https://derekmichaelwright.github.io/AGILE_LDP_UAV/LDP_UAV_Vignette.html.

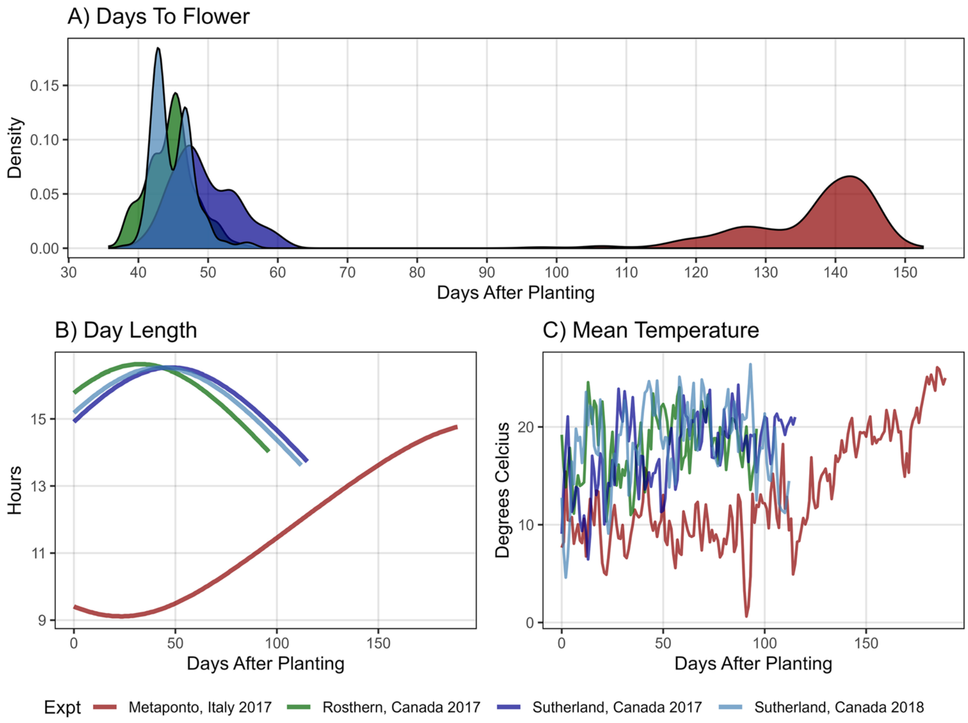

FIGURE 1 Phenological and environmental data from the field trials grown in Metaponto, Italy, 2017 (It17; planted November 28); Rosthern, Canada, 2017 (Ro17; planted May 4); and Sutherland, Canada, 2017 and 2018 (Su17 and Su18; planted May 4 and 9, respectively). (A) Days from sowing to flower, (B) day length, and (C) mean daily temperature.

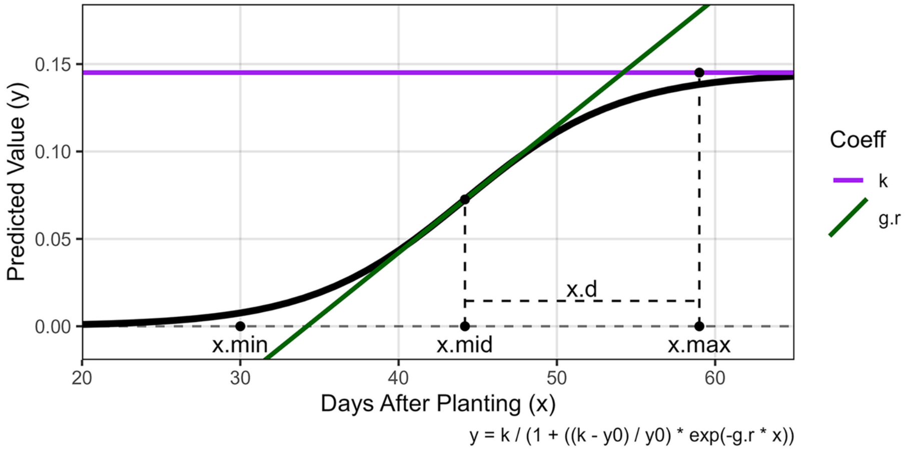

FIGURE 2 Modeled growth curve highlighting some derived coefficients and additional traits, which can be calculated. k, maximum value; g.r, growth rate for the linear portion of the growth phase; x.min, time at which 5% of growth occurs; x.mid, time at which 50% of growth occurs; x.max, time at which 95% of growth occurs; x.d, doubling time for growth (defined as x.max–x.mid).

2.4 | Genotyping and genome-wide association analyses

Genotyping of all 324 accessions of the LDP was done using a custom lentil exome capture assay described by Ogutcen et al. (2018). Genotypic data output as a high-confidence SNP array was accessed from https://knowpulse.usask.ca/experiment/AGILE-Exome-targeted-Re-sequencing. Markers that were not biallelic or had >20 accessions with missing data, >20% heterozygosity, or a minor allele frequency <5% were removed prior to analysis. The remaining 267,845 SNPs with high coverage sites across the lentil genome were used in a genome-wide association study (GWAS).

GWAS were performed using a mixed linear model (Yu et al., 2006), fixed and random model circulating probability unification (Liu et al., 2016), and Bayesian-information and linkage disequilibrium iteratively nested keyway (Huang et al., 2019) implemented in the genome association and prediction integrated tool in R (Wang & Zhang, 2021).

3 | RESULTS AND DISCUSSION

In lentil, crop adaptation results from a combination of multiple traits, largely driven by phenology and plant growth and structure. Achieving adequate yield requires not only that the crop flower at an appropriate time in a given environment but also that it have the appropriate growth habit to achieve one or more of the following: to suppress/outcompete weeds through vigorous growth during the early post-emergence stage, build up biomass (source) during the vegetative growth phase to drive seed yield (sink), and be suitable for mechanical harvesting (via increased height). We leverage existing trials (Wright et al., 2021) by imaging them from UAVs to investigate the effect of genotype and environment on growth of a diverse panel of lentils. We then used associated genotypic data to better understand the genomic regions involved.

3.1 | Temporal data derived from UAV images can be used to model growth curves

Using UAV-captured image-derived data, we estimated plot height, area, and volume of lentil at multiple time points across site-years in Italy and Canada (Figure S1). As previously observed for lentil (Nielsen et al., 2022), these image-derived traits show reliable correlations with those of manually measured canopy height and biomass (Figure S2). The correlations were less robust for the Italian site partially due to the flights not corresponding exactly to the same date as the ground-truth measurements as well as having less reliable ground-truth data as compared to our Saskatchewan field trials, which were phenotyped with the intention of ground-truthing in mind.

Although there are some practical limitations with regard to the implementation of UAVs within breeding programs, such as the cost of UAV equipment and software, training for operators, regulatory restrictions, etc., manual measurements of crop growth are often limited to easily measurable traits such as plot height, whereas traits such as plot area or volume can be difficult or impossible to measure in an objective or non-destructive way. Using temporal data derived from repeated imaging, we were able to model growth curves based on plot height, plot area, and plot volume for each accession within the LDP across 3 of the 4 site-years of image-derived data (Figure 3). For the Su18 site-year, a lack of sufficient data points during the early growth phase resulted in inaccurate modeling of growth curves, emphasizing the need to have sufficient data throughout all key growth phases to accurately model growth—something that would be even more difficult without image-derived data. By modeling growth curves for plot height, area, and volume, we were able to extract additional phenotypes from the data sets. Coefficients derived from the modeling equation (Figure 2) can be used as derived phenotypes. Furthermore, it is possible to calculate additional, novel phenotypes, which incorporate other related traits, such as the percentage of total volume achieved by the date of flowering. These approaches helped expand the analysis and enhance our understanding of complex traits such as crop growth across environments, demonstrating the utility of using UAV-captured imagery to derive data for modeling growth in lentil. Despite the initial challenges of implementing UAV systems within breeding programs, our results showcase the potential for using UAV-derived imagery to phenotype large, diverse lentil populations for multiple growth-related traits.

3.2 | Environmental conditions differentially influence the growth of lentil accessions

Environmental conditions, predominantly temperature and photoperiod, influence the phenology of lentil accessions. Large variation exists in sensitivity to both temperature and photoperiod within the LDP (Wright et al., 2021). Additionally, GWAS for phenology-related traits across multiple environments with this population has shown that these processes are driven by underlying genes that interact differently depending on the environment in which they are grown (Neupane et al., 2022). As a result, we hypothesized that growth rate and crop architecture would also have G × E interactions, potentially driven by factors including mean temperature and photoperiod during the growing season.

Significant G × E interactions were observed for all growth-related traits derived from the growth curve modeling of plot height, area, and volume, and in some cases accounted for >20% of the trait variance (Figure S3). Comparisons of the growth curves across environments demonstrate the strong effect of the environment on growth, with all accessions in Ro17 and Su17 essentially completing their growth before most accessions started their rapid growth phase in It17 (Figure 3). This is driven in large part by the Italian trial being grown during the winter-spring when temperatures and day length are lower than what is experienced at the Canadian locations. As a result, larger variations in plant structure, plot height, and area were observed in It17 than in Ro17 and Su17 (Figure 4A). In addition, we can observe differences between accessions that are strongly influenced by environmental conditions versus others, which are not, and chose to highlight four accessions, which demonstrate this in Figure 3. For example, CDC Rosetown AGL and ILL 5888 AGL show a strong genetic component to growth, being always large or

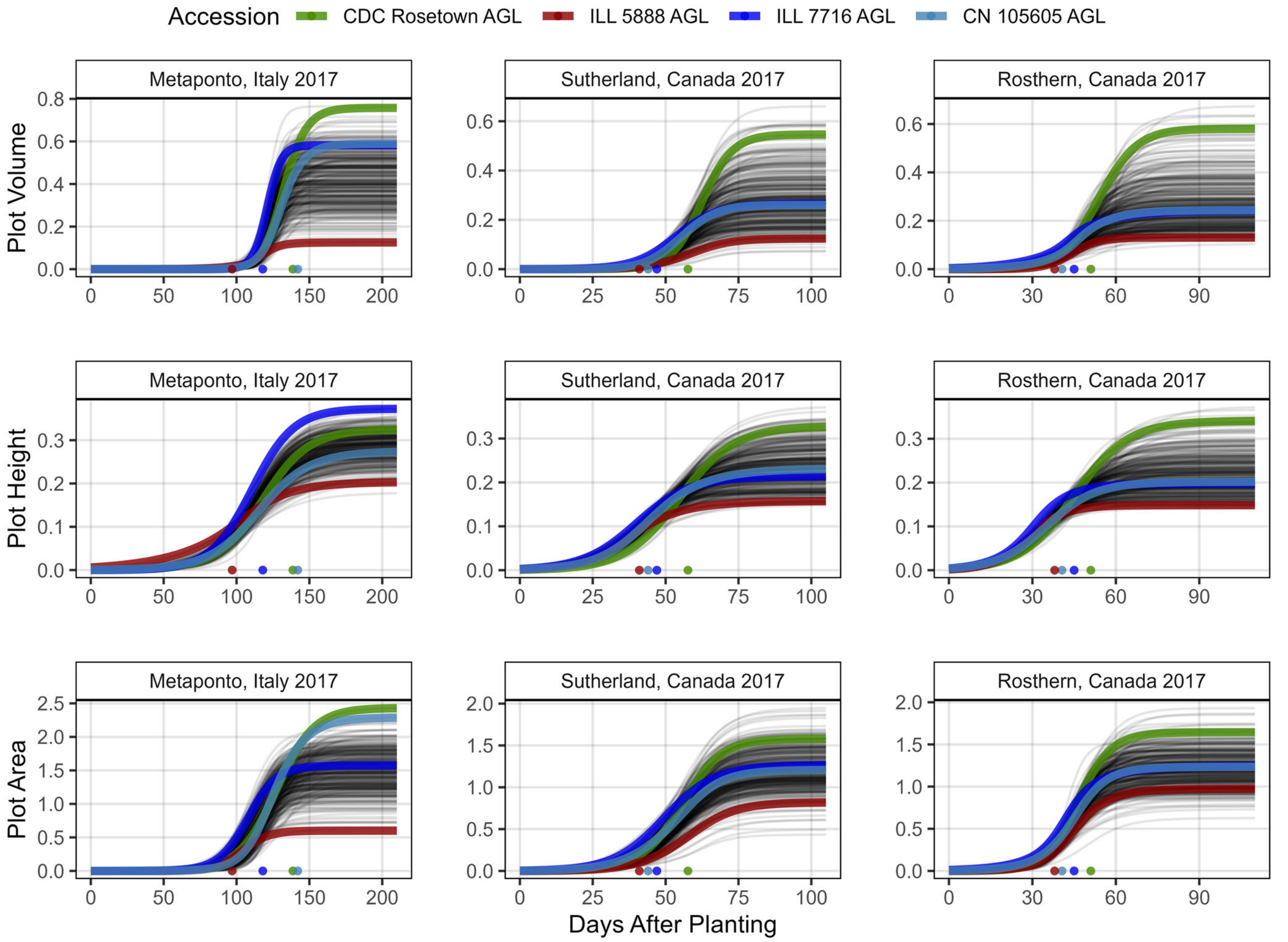

FIGURE 3 Growth curves for plot volume, height, and area for each accession within the lentil diversity panel grown in 2017. Four contrasting accessions are highlighted in color with corresponding dots indicating the day flowering started (after planting). Metaponto, Italy, was planted November 28, while Sutherland and Rosthern, Canada, were planted May 4 and 19, respectively.

small, respectively, regardless of the environment. In contrast, accessions ILL 7716 AGL and CN 105605 AGL show strong G × E interactions influencing crop growth. In Ro17 and Su17, these two accessions behave similarly in plot height and area, both displaying medium height and area. In It17, however, these two accessions behave very differently, with ILL 7716 AGL becoming tall and thin, while CN 105605 AGL grew relatively short and wide.

In Ro17, Su17, and Su18, spring-sown environments, where mean temperatures and photoperiods are relatively high (Figure 1B,C), adaptation is driven by late flowering, which should allow the plants to build up biomass before entering their reproductive period. It is commonly assumed that later flowering lines grow more biomass, which allows for more energy to be diverted into seed mass during the reproductive phase, resulting in higher yield, but this is not always true (Figure 4B,C). A study of 287 lentils grown in the US Pacific Northwest also found correlations of biomass with seed yield (Tullu et al., 2001); however, fall-sown field trials with eight accessions grown in multiple locations in Australia showed the opposite: earlier maturing accessions with shorter vegetative periods yielded more than the later maturing accessions (Maphosa et al., 2023), trends which are also observed in South Asian environments (Kumar et al., 2012, 2016). In It17, relatively low mean temperatures and photoperiods (Figure 1B,C) resulted in G × E interactions that affected flowering time (Figure 1A; Wright et al., 2021), growth, and yield (Figure 4) in complex ways. In contrast to the temperate environments (Ro16, Su17, and Su18), volume and yield appeared to be decoupled in It17, which could partially explain the lower reliability of the UAV imagery from It17. Unlike Ro16, Su17, and Su18, plot volume had a higher correlation with straw biomass than total biomass (Figure S2B,C). Further illustrating this G × E effect on growth and yield, we looked specifically at accessions originating from three contrasting environments with multiple entries—Canada, Iran, and India (Figure 4). Accessions originating from Canada had low yields in It17 but high yields

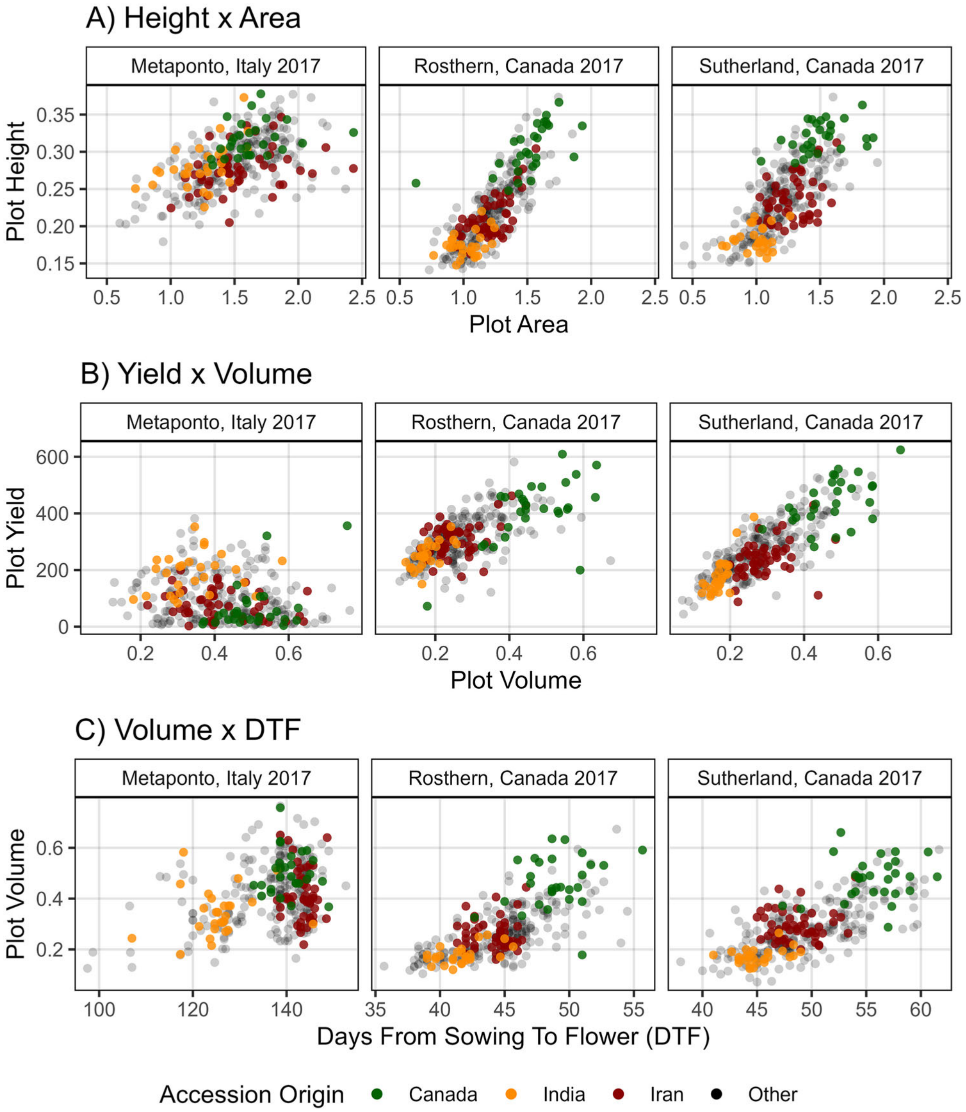

FIGURE 4 Correlations of (A) plot area versus plot height, (B) plot volume versus plot yield (grams of seed/plot), and (C) days from sowing to flower (DTF) versus plot volume across 3 site-years. Plot height, area, and volume are all derived from growth curve modeling. The color of dots represents the country of origin for each accession.

in Ro17 and Su17, despite having a large size (plot height, area, and volume) in all environments. In contrast, accessions originating from India had smaller sizes in all environments but yielded more in It17 compared to accessions originating from Canada. Accessions originating from Iran yielded more in Ro17 and Su17 compared to It17 despite having a larger size in It17, likely attributable to their phenological (DTF) response in each environment. This coupling/decoupling of yield and volume of lentil based on the environmental conditions has been observed previously (Lake & Sadras, 2021) and reinforces the notion that adaptation is a complex trait, driven by different factors in different environments, and there are likely multiple approaches for achieving adaptation in a crop species. It is important to understand how the growth of a particular genotype contributes to its success in each environment. Our results showcase contrasting genotypic responses to different environments and emphasize the need for breeders to use caution if making inferences about growth patterns based on data from other locations.

3.3 | Cluster analysis enables the identification of different growth strategies among lentil accessions

A PCA of data derived from image-derived growth curve modeling for plot height, area, and volume along with hierarchical clustering was used to identify eight cluster groups (Figure S4). In order to avoid bias due to unequal number of sites from each macroenvironment, we chose to only use data

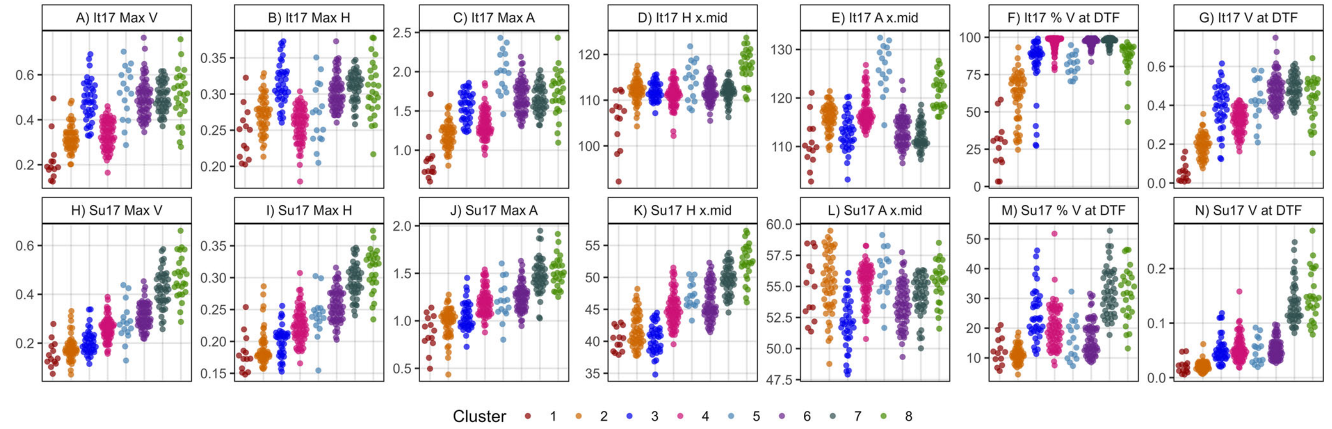

FIGURE 5 Values derived from growth curve modeling of plot height, area, and volume in Metaponto, Italy, 2017 (It17) and Sutherland, Canada, 2017 (Su17), for the lentil diversity panel, colored by cluster group. (A and H): Max V, maximum plot volume based on *k* values from the modeling of plot volume over the growing season. (B and I): Max H = maximum height based on *k* values from the modeling of plot height over the growing season. (C and J): Max A = maximum area based on *k* values from the modeling of plot area over the growing season. (D and K): H x.mid = day after planting when half of maximum plot height is achieved. (E and L): A x.mid = day after planting when half of maximum plot height is achieved. (F and M): % V at days from sowing to flower (DTF) = the percent of maximum plot volume achieved at the start of flowering. (G and N): V at DTF = plot volume at the start of flowering.

from the Su17 and It17 site-years (Figure S5). These cluster groups best represent the different growth patterns within the LDP across two contrasting macroenvironments (Figure 5). Clusters 1 and 2 had the lowest plot volumes regardless of their environment (Figure 5A,H), although cluster 2 was slightly larger in volume and flowered later than cluster 1 during its growth phase in It17 (Figure 5F). In Su17, all cluster groups flowered around or before plot volume reached 50% of its maximum. In It17, however, flowering tended to occur toward the completion of crop growth for most clusters (Figure 5F,M and 6C). Members of clusters 1 and 2 were exceptions, which flowered earlier during crop growth in both environments. Comparing clusters 3 and 5, the growth habit of cluster 3 can be described as tall and thin in It17, the opposite of cluster 5, which was short and wide (Figure 5B,C). These differences were not observed in Su17 (Figure 5 I,J). Cluster 3 had large plot volume in It17 but small plot volume in Su17 (Figure 5A,H), attributable to a lower plot height in Su17 compared to It17 (Figure 5B,I). Cluster 4 had medium plot height, area, and volume in both environments (Figure 5A–C,H–J). Clusters 6, 7, and 8 had similar plot height, area, and volumes in It17 (Figure 5A–C); however, cluster 6 was smaller, earlier to start growth, and smaller in volume at the time of flowering, and had achieved less percent maximum growth prior to flowering in Su17 (Figure 5H–K,M,N). Cluster 8 had among the highest plot volumes in both It17 and Su17, and the latest date at which half of plot height was reached (Figure 5A,H,D,K).

Unlike the PCA based on days from sowing to flowering in this population where the first three principal components accounted for 89.7% of variability (Wright et al., 2021), the first three principal components based on growth parameters only accounted for only 65.5%–41.3%, 14.4% and 9.8%, respectively, of the variation (Figure S4). This indicates that, unlike flowering time, differences in crop growth in lentils are influenced by a more complex set of environmental parameters and interactions. For most accessions in this study, plot height and area were larger in It17 than Su17 (Figure 6A,B); however, accessions exhibiting all other possibilities were also present: taller and thinner in It17 than Su17, shorter and wider in It17 than Su17, and accessions with larger plot height and area in Su17 than It17. In addition, the amount of change in plot height and area between It17 and Su17 varied depending on the cluster group (Figure 6A). This clustering approach helped reveal differing growth patterns among diverse lentil genotypes and across varying environments. Furthermore, the addition of growth-related data on this population from other environments could help refine the clustering and further explain growth patterns in lentil.

3.4 | Genome-wide association studies identify novel growth-related QTL and potential markers for selecting in different environments

GWAS analyses of growth-related traits in this study revealed numerous QTL associated with crop growth in lentil (Figure 7; Table S1). It was possible to identify significant associations with manually collected and image-derived traits. The addition of phenotypes derived from the modeling of growth curves reinforced these QTL and added new ones. To identify regions of the genome playing an outsized role in crop growth, and to help account for false positives due

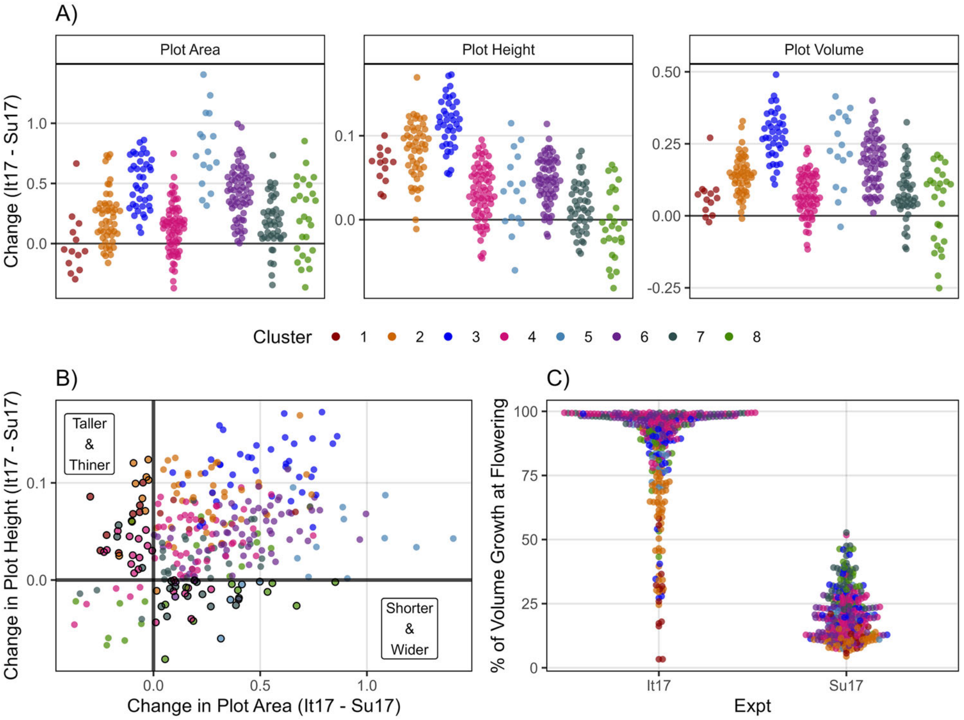

FIGURE 6 (A) Differences in plot height, area, and volume for individuals grown in Metaponto, Italy, 2017 (It17) versus in Sutherland, Canada, 2017 (Su17), separated by cluster. (B) Change in plot height versus plot area, highlighting (black outline) accessions, which were either taller and thinner in It17 versus Su17 or shorter and wider in It17 versus Su17. (C) percentage of maximum plot volume achieved at the start of flowering.

to the high number of traits analyzed, we plotted the number of significant associations within 1 Mbp increments across the genome (Figure 7). Using manual and image-derived phenotypes, we identified QTL/markers, which showed a strong effect on plot height in Su17 but not It17, and vice versa (Figure 8A,B), allowing for the development of environment-specific markers for selection, and demonstrates the G × E interactions influencing crop growth in lentil. In addition, we found QTL/markers associated with crop growth regardless of the environment (Figure 8C). While the reliability of the Italian image-derived data were somewhat questionable due to lower correlations with our manually collected measurements than in our temperate environments (Figure S2), GWAS results of the Italian data did yield significant associations common between all environments (Figure S6). Although associations with crop height could be identified with just manually collected traits, by incorporating traits derived from our UAV images and modeling of growth curves, we were also able to identify association with plot area and volume (Figure S7), something not possible to determine with only the manually measured phenotypes. Tefera et al. (2022) demonstrated the ability to estimate early season growth (vigor) in pea using images derived from UAVs. In our study, we were able to identify associations with early growth (Figure 8D), represented by the x.min coefficients, using image-derived traits and growth curve modeling, suggesting it may be possible for breeders to select for early crop growth and potentially more weed competitive lentil varieties. Further experiments will be needed to validate these associations and identify potential causal genes within these QTL.

Crop growth and plant architecture influence and are influenced by other agronomically important traits. Tefera et al. (2022) found that early season growth (vigor) in pea had positive or negative correlations with seed yield depending on the environment. Lowest pod height, a trait important for mechanical harvestability, is being investigated in many legume species (Kuzbakova et al., 2022). In Australian lentil breeding programs, selection for yield has generally resulted in increased plant height and a reduced number of branches (Silva-Perez et al., 2022). As expected, our GWAS of crop growth parameters also associated with QTL previously linked with flowering time in lentil (Figure 7; Neupane et al., 2022), in line with our current understanding of adaptation in lentils. In temperate environments (Ro17 and Su17), there is a tight correlation between plot volume and yield, and accessions with early flowering alleles tended to be smallest in volume and lowest in yield, partially due to simultaneous selection within temperate breeding programs for later flowering and high yield. For example, all the accessions within the LDP that originate from Canada contain the *FTb* allele for late flowering (Haile et al., 2021; Neupane et al., 2022). With this information, it is possible to make selections based off of flowering alleles and growth habit when attempting to increase genetic diversity (Figure S8), an important factor for

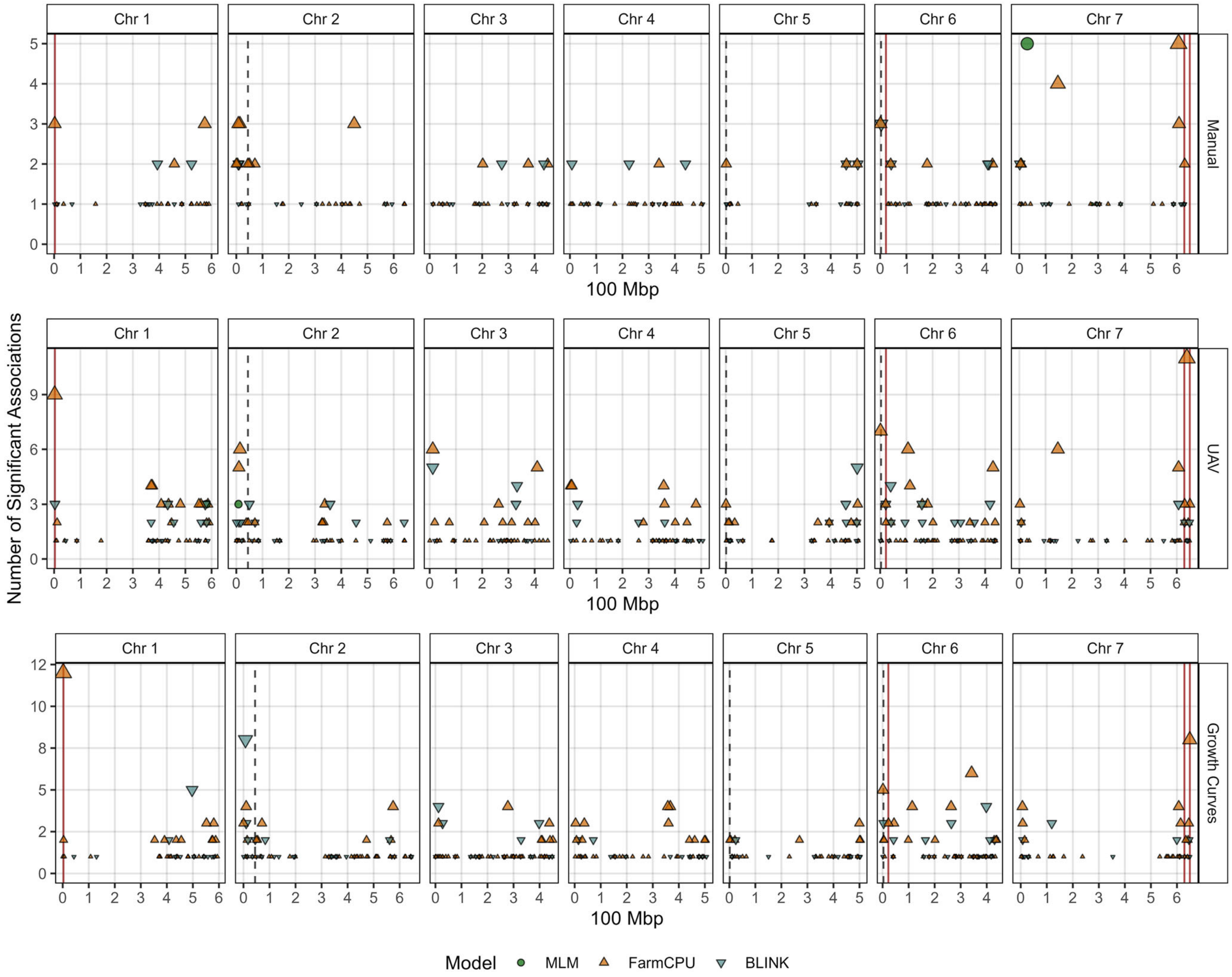

FIGURE 7 The number of significant associations identified in 2 Mbp windows across the lentil genome. The top panel includes only manually collected traits, the middle panel includes only traits phenotyped using unmanned aerial vehicles (UAV), and the bottom panel includes only traits derived from growth curve modeling. Vertical lines represent specific base pair locations to facilitate comparisons across results, with the dashed black vertical lines representing phenology-related QTL identified in this population in a previous study (Neupane et al., 2022), while the solid red vertical lines represent four novel QTL identified herein (Lcu.1GRN.Chr1p2884425, Lcu.1GRN.Chr7p628672781, Lcu.1GRN.Chr6p22131416, and Lcu.1GRN.Chr7p649509676) for further discussion.

ensuring breeders can continue to make progressive genetic gains. However, it should be noted that differing strategies may be needed in different regions. For example, Lake et al. (2024) found that most Australian lines have the *FTb* allele for early flowering, and the degree to which biomass correlated with yield was dependent on the environment.

4 | CONCLUSIONS

By using temporal, UAV-derived data, we modeled growth curves and derived phenotypes and identified associations demonstrating the usefulness of UAV imagery-derived data for use by plant researchers and breeders. In comparison with manually collected growth traits, UAV imagery-derived data added more dimensionality to analyses by introducing both plot area and volume as traits and enabled the high-throughput collection of data over the entire duration of a field trial. Our results highlight the diversity of growth habits within cultivated lentil, as well as the strong G × E interactions that occur across different environments, improving our understanding of adaptation in lentil. By performing a PCA and hierarchical clustering of various phenotypes derived from growth curves across different macro-environments, we were able to dissect the various growth patterns exhibited within the LDP. Running GWAS on all of these growth-related traits identified QTL/markers, which can be used to help identify genes involved in crop growth and aid breeders in selection for desired growth habit and/or increase genetic diversity within their material.

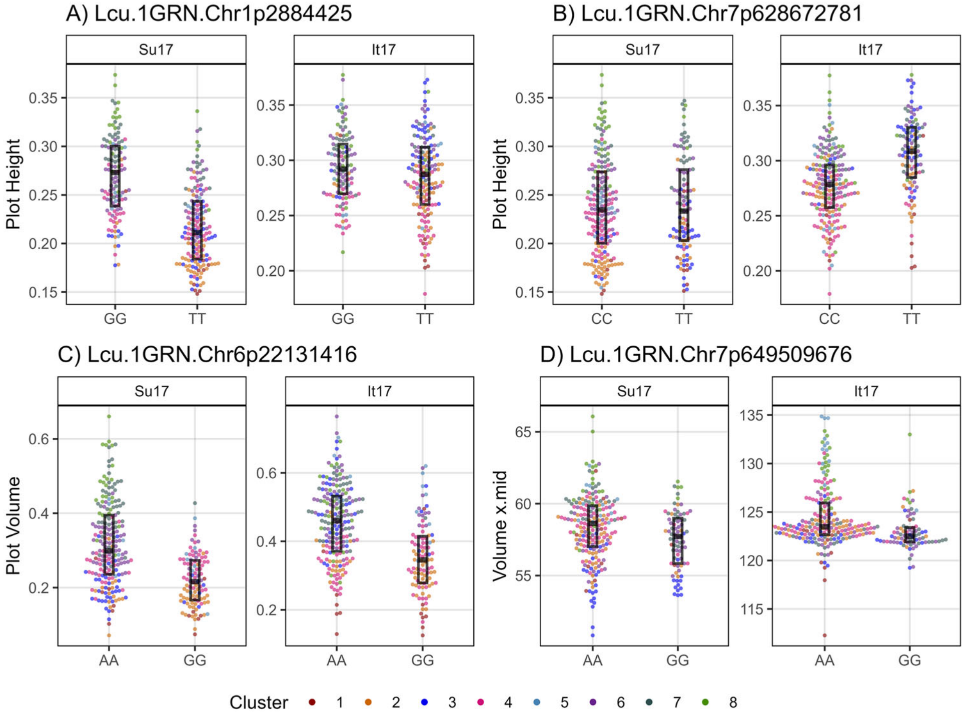

FIGURE 8 Allelic effects of selected markers in Sutherland, Canada, 2017 (Su17) and Metaponto, Italy, 2017 (It17). (A) Lcu.1GRN.Chr1p2884425 and (B) Lcu.1GRN.Chr7p628672781 on plot height. (C) Lcu.1RBY.Chr6p22131416 on Plot Volume. (D) Lcu.1GRN.Chr7p649509676 on volume x.min (time at which 50% of plot volume growth occurs). Values for plot height and volume were derived using the *k* coefficient from the growth curve modeling.

AUTHOR CONTRIBUTIONS

Derek M. Wright: Data curation; formal analysis; investigation; visualization; writing—original draft; writing—review and editing. **Sandesh Neupane**: Formal analysis; investigation; visualization; writing—original draft; writing—review and editing. **Steve Shirtliffe**: Conceptualization; funding acquisition; project administration; resources; supervision; writing—review and editing. **Tania Gioia**: Data curation; investigation; resources; supervision; writing—review and editing. **Giuseppina Logozzo**: Investigation. **Stefania Marzario**: Investigation. **Karsten M. E. Nielsen**: Investigation; writing—review and editing. **Kirstin E. Bett**: Conceptualization; data curation; funding acquisition; methodology; project administration; resources; supervision; visualization; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available online at https://knowpulse.usask.ca/research-experiment/AGILE-UAV and https://github.com/derekmichaelwright/AGILE_LDP_UAV or from the authors upon request.

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