

Methods

**AIRMEASURER**: open-source software to quantify static and dynamic traits derived from multiseason aerial phenotyping to empower genetic mapping studies in rice

Gang Sun1*, Hengyun Lu2*, Yan Zhao2*, Jie Zhou1, Robert Jackson3, Yongchun Wang2, Ling-xiang Xu1, Ahong Wang2, Joshua Colmer4, Eric Ober3, Qiang Zhao2, Bin Han2 and Ji Zhou1,3

1State Key Laboratory of Crop Genetics & Germplasm Enhancement, Academy for Advanced Interdisciplinary Studies, Jiangsu Collaborative Innovation Center for Modern Crop Production Co-sponsored by Province and Ministry, Nanjing Agricultural University, Nanjing, 210095, China; 2National Center for Gene Research, CAS Center for Excellence in Molecular Plant Sciences, Chinese Academy of Sciences, Shanghai, 200233, China; 3Cambridge Crop Research, National Institute of Agricultural Botany (NIAB), Cambridge, CB3 0LE, UK; 4Earlham Institute, Norwich Research Park, Norwich, NR4 7UH, UK

Authors for correspondence:
Ji Zhou
Email: ji.zhou@njau.edu.cn, ji.zhou@niab.com

Bin Han
Email: bhan@ncgr.ac.cn

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Summary

- Low-altitude aerial imaging, an approach that can collect large-scale plant imagery, has grown in popularity recently. Amongst many phenotyping approaches, unmanned aerial vehicles (UAVs) possess unique advantages as a consequence of their mobility, flexibility and affordability. Nevertheless, how to extract biologically relevant information effectively has remained challenging.
- Here, we present AIRMEASURER, an open-source and expandable platform that combines automated image analysis, machine learning and original algorithms to perform trait analysis using 2D/3D aerial imagery acquired by low-cost UAVs in rice (Oryza sativa) trials.
- We applied the platform to study hundreds of rice landraces and recombinant inbred lines at two sites, from 2019 to 2021. A range of static and dynamic traits were quantified, including crop height, canopy coverage, vegetative indices and their growth rates. After verifying the reliability of AirMeasurer-derived traits, we identified genetic variants associated with selected growth-related traits using genome-wide association study and quantitative trait loci mapping.
- We found that the AIRMEASURER-derived traits had led to reliable loci, some matched with published work, and others helped us to explore new candidate genes. Hence, we believe that our work demonstrates valuable advances in aerial phenotyping and automated 2D/3D trait analysis, providing high-quality phenotypic information to empower genetic mapping for crop improvement.

Introduction

Rice (*Oryza sativa*) is one of the key staple foods, feeding >50% of the global population (Muthayya *et al.*, 2014). Breeding for rice improvements in yield potential, grain quality and resistance to stresses is vital for its climate-change adaptation and, thus, food security in many rice-consuming nations (Nakashima *et al.*, 2007; Jagadish *et al.*, 2012). This relies on selecting favourable phenotypes of agronomic traits from thousands of varieties in the field, which in turn heavily relies on specialists’ visual assessment (Bevan *et al.*, 2017; Roitsch *et al.*, 2019). To help accelerate the selection procedure, many field-based phenotyping approaches have been introduced (Zhao *et al.*, 2019; Yang *et al.*, 2020).

Additionally, as agronomically important traits are controlled by the expression of multiple genes and modulated by the environment, phenotyping can assist researchers to understand underlying biological mechanisms that contribute to genetic gain (Hartung & Schiemann, 2014; Furbank *et al.*, 2019). Through genome-wide association studies (GWAS), the genetic architecture of some agronomic traits in rice has been dissected (Huang *et al.*, 2010; Yang *et al.*, 2014; Tang *et al.*, 2019), laying the foundation of identifying functional diversity of alleles to discover valuable genes (Xing & Zhang, 2010). These contributions have led to advances in rice genetics and the development of new varieties with desired qualities, including high yield potential,

*These authors contributed equally to this work.
resistance to stresses and increased resource-use efficiency (Barabaschi et al., 2015; Du et al., 2018; Li et al., 2018).

Certain traits such as plant height can be phenotyped at a specific time point; however, for growth- and yield-related traits that are genetically complex and influenced heavily by environmental factors, their phenotypes need to be examined dynamically (Naito et al., 2017; Mu et al., 2022). Nevertheless, to achieve this target, consistent data collection and trait analysis are required, which has posed significant challenges in developing reliable solutions for practical breeding programmes and field-based plant research (Shakoor et al., 2017; Pieruschka & Schurr, 2019). In essence, several problems need to be addressed, including: (1) scalability, trials are normally large-scale and at multiple sites; (2) affordability, resources are usually limited and solutions need to be cost-effective; (3) accuracy and repeatability, analysis results should be consistent and reproducible in other trials; (4) processing cycle, the duration between breeding cycles or multiseason experiments is often brief, requiring data to be processed, analyzed and fed-back promptly to enable timely decisions (Großkinsky et al., 2015; Atkinson et al., 2018). Recently, several advances have been adopted by breeders and plant researchers, but many attempts remain at early stages (White et al., 2012; Juliana et al., 2019). New tools derived from some academic research have often worked at relatively small scale and with limited accessibility as a result of bespoke hardware, proprietary software and specialized packages, preventing them from being employed easily (Yang et al., 2020, 2021). Furthermore, to exploit genomic resources, traits of interest and genetic diversity need to be assessed across sites and seasons, demanding accessible data collection and analysis toolkits (Naito et al., 2017; Atkinson et al., 2018). Hence, methodological advances shall intend to address the above challenges, which is the emphasis of this study.

One of the most exciting advances recently was the rapid development of unmanned aerial vehicles (UAVs, also known as unmanned aircraft systems) and their applications in crop monitoring resulting from their mobility, throughput and affordability (Shi et al., 2016; Maimaitijiang et al., 2017; Jang et al., 2020). There are numerous examples in the literature reporting UAV-based phenotyping using sensors such as red-green-blue (RGB) cameras, multi- and hyperspectral devices, Light Detection and Ranging (LiDAR), and thermal and infrared sensors (Kachamba et al., 2016; Gracia-Romero et al., 2017; Harkel et al., 2020; Hyvppä et al., 2020). Some work also reported quantitative trait loci (QTL) mapping of traits including plant height and vegetation fraction (Hassan et al., 2019; Wang et al., 2019; Ogawa et al., 2021). Nevertheless, most of these studies focused on estimating static traits collected at specific time points (Shakoor et al., 2017; Rodene et al., 2022), which often missed the dynamic nature of plant growth and development. Key agronomic traits (e.g. senescence and stem elongation) vary in time and space, which require new approaches to collect and analyse (Xu et al., 2018; Anderson et al., 2020). In fact, in a trial containing diverse genotypes, each line grows at a different pace, and thus dynamic analysis can provide meaningful comparisons between the genotypes (Hartung & Schiemann, 2014). Finally, changing behaviours of target traits, within or across seasons, can characterize the plant’s complex responses to external stimuli, which are direct evidence to reveal spatial and temporal changes in the expression of genes and their regulators (Roitsch et al., 2019; Mu et al., 2022).

To extract meaningful information from UAV-collected imagery, many analytic solutions have been developed to measure traits related to yield, stress tolerance and growth patterns, using morphological, spectral and textural properties (Perez-Sanz et al., 2017; Jiang et al., 2021), most of which have focused on dryland crops. For example, EASY MPE (Tresch et al., 2019) combined excess green (ExG) and automatic thresholding to study soybean; AIRSURF (Bauer et al., 2019) employed deep learning to count and classify lettuces; GRID (Chen & Zhang, 2020; Tang et al., 2021) utilized pixel-wise K-means clustering to delineate irregular (e.g. zigzag) or regular (e.g. grid-based) trial layouts for wheat trials; RUAS::PLOTSHPCREATE (Anderson & Murray, 2020) created polygon shapefiles using parameters (e.g. field direction and plot size) to study maize; FIELDIMAGER (Matias et al., 2020) incorporates manual inputs (e.g. row and column numbers) into the extraction of plot-based traits for potato.

Still, limited tools are available for nonexperts to examine multigenic traits and develop markers for paddy field crops (e.g. rice), which are complex as a consequence of changing water levels (e.g. resulting from rainfall and draining) and many voluntary plants (e.g. duckweed) compared with dryland crops (Ogawa et al., 2021). Moreover, few research groups have the resources to process large-scale aerial images, or to develop complex algorithms to address problems in automated trait analysis (Roitsch et al., 2019; Zhu et al., 2021). Hence, along with the development of open-source computer vision, machine learning and data science libraries (Howse, 2013; Virtanen et al., 2020), open solutions will be valuable to equip plant researchers with new toolkits to study complicated crops.

In order to address some of the challenges, we have developed AirMeasurer, an open-source platform that automates trait analysis for rice trials using 2D orthomosaics and 3D point clouds acquired by low-cost UAVs. First, we established tailored protocols for regular flight missions and data pre-processing. Secondly, varied 2D/3D analysis algorithms were integrated into the platform to quantify static traits such as seedling number, plant height, canopy coverage and vegetative indices, using morphological, spectral and textural signals. Thirdly, we developed an original algorithm to compute dynamic traits based on static traits, including growth rates of the target traits and their rapid growth phases, which were time-consuming or impossible to score previously. To ensure that our work could reach the broader research community, we created a graphical user interface (GUI) for non-experts to use. Finally, to validate the platform and its utility in research, we applied the AirMeasurer-derived traits collected from hundreds of rice landraces and recombinant inbred lines (RILs) in a multiseason case study (2019–2021) to genetic mapping studies (i.e. GWAS and QTL mapping) and identified reliable loci.
Materials and Methods

Plant materials and field experiments

In order to develop a UAV-based imaging protocol for multisite phenotyping, we established two experiments (2019–2021; Supporting Information Fig. S1a): (1) one focused on 254 landraces (Huang et al., 2012) in Shanghai (the 2019/2020 seasons), including 103 japonica, 40 intermedia and 111 indica types; (2) the other studied 191 homozygous RILs in Hainan (the 2020/2021 seasons), derived from the crossing parents Nipponbare (Oryza sativa ssp. Japonica) and 93–11 (Oryza sativa ssp. Indica), two popular varieties (Huang et al., 2010). In 2019, 177 RILs were used for manual assessment as a consequence of agronomic issues with some RILs during grain-filling. The sites were chosen owing to their geography and weather conditions. Crops at both sites were managed using standard husbandry and agronomic inputs according to local conditions. Landraces (Fig. S1b) and RILs (Fig. S1c) were sown in 2 × 1.1 m plots, 18 plants per plot. To maximize the efficient use of field space and facilitate initial selection (Payne, 2006), we did not introduce plot-level replicates; however, the same lines were repeatedly used in this multiseason case study. Details of the trial design, plant materials and geo-locations are provided in Notes S1.

Ground truthing

In-field ground truth measurements to validate AIRMEASURER-derived traits were conducted by field workers. Maximum plant height was measured with a metre ruler in the late reproductive phase. After grain-filling, six plants in a plot were straightened and the distance from ground level to the top of rice spikes was measured. Heading date was scored manually, when there were five plants with panicles emerging 25 mm above the flag leaf sheath. To verify traits such as ExG and canopy coverage used for dynamic trait analysis, images of 29–30 randomly selected plots at six growth stages between early vegetation and early ripening (177 plots in total) were analyzed manually using the Fiji/ImageJ software (Schindelin et al., 2012), through which plot-based green-channel intensity values (0–255; measured from linear histogram) and canopy coverage (in pixels; using the auto-thresholding function) were obtained. To validate AIRMEASURER-derived plant height at different growth stages, technicians manually measured calibrated 3D point clouds (with unwanted terrain features removed) to obtain plot-level canopy height at eight time points throughout the 2019 season (177 per point, 1416 in total).

Workflow of UAV-based phenotyping

When carrying out aerial phenotyping, we implemented a four-step workflow (Fig. 1a): (1) experiment setups – including trial design (e.g. field layouts, target traits) and ground control points (GCPs; Figs 1b, S1d); (2) aerial imaging – providing guidelines to pilots to execute flight plans (Figs 1c, S1e); (3) data pre-processing – producing 2D field-level orthomosaic images (in TIFF) and 3D point cloud files (in LAS) from acquired aerial images; (4) automated trait analysis using a combination of 2D/3D image processing, spectral analysis, and machine learning techniques to perform plot segmentation and plot-based trait analysis using morphological, spectral, and textural signals (all the traits produced by AIRMEASURER are listed in Table 1).
images using the Pix4DMapper software (Pix4D, Lausanne, Switzerland; Fig. 1d); and (4) phenotypic analysis – combining spectral, textural and morphological properties of plants to perform automated trait analysis (Fig. 1e).

Aerial imaging using low-cost UAVs

At each site, in-field settings (Fig. 1b) such as GCPs, height reference panels, spectral reflectance mats, or real-time kinematic positioning (RTK) were applied according to recommended practices published previously (Watanabe et al., 2017). To ensure that the imaging protocol could be adopted easily, we chose to use low-cost drones (e.g. Mavic 2 Pro; DJI, Shenzhen, China). Because smaller UAVs generated less downdraft, they could limit wind disruption of plant canopies during the low-altitude imaging. We designed two mission plans: (1) field-level imaging (25–35 m altitude), collecting RGB images speedily to limit colour distortion caused by natural illuminance (e.g. Fig. 1b left); (2) plot-level imaging, conducting flights with tailored flight parameters at low altitudes (10–15 m). Flights were normally carried out 10–12 times per season, among which eight flights were selected for time series measures (detailed mission plans, imaging protocols and guidelines are included in Notes S2).

3D point cloud processing and canopy height model

There can be unwanted noise in 3D point clouds generated by the Structure-from-Motion (SfM) algorithm (Singh & Frazier, 2018). To measure morphological features reliably (Fig. 2a), we first denoised the SfM-generated 3D points (e.g. for a 0.1-ha field, low-density 3D reconstruction could produce >30 million points). Second, we implemented the Statistical Outlier Removal (SOR) algorithm (Hodge & Austin, 2004) to remove outliers (red-coloured points, Fig. 2b). Third, a ground-level filter was developed based on the Cloth Simulation Filter (CSF) algorithm (Zhang et al., 2016), classifying denoised 3D points into ground-level and aboveground groups. Because the CSF was designed for ultra-large land surveillance, we optimized the filter by reducing its grids and nodes (Fig. 2c). Finally, we removed unwanted terrain features (e.g. the field-level slope) using geo- or 3D-coordinates recorded from GCPs (saved in a shapefile, SHP). The procedure to correct geometric distortion is included in Notes S2 and S3, which shows the improved height measurements after removing field-level slopes.

Next, we generated a digital surface model (DSM, i.e. ground-level points) and a digital elevation model (DEM, i.e. aboveground points) using the LidarTinGridding function (Lindsay, 2016) in WHITEBOXTOOLS (Fig. 2d). We defined a region of interest (ROI) according to the SHP file (red markers, Fig. 2e). The DSM was subtracted from the DEM to retain aboveground plant information, resulting in a canopy height model (CHM) representing plant spatial signals with greyscale values (i.e. the brighter a pixel, the higher the point). Finally, we combined the CHM with spectral signals using the getPerspectiveTransform function (Mezirow, 1978) in OpenCV, which realigned the CHM (Fig. 2f, upper) with the field-level orthomosaic (Fig. 2f, lower). Spatial features were pseudo-coloured (Fig. 2g, upper), ranging from 0 (dark blue, for bare plots) to 150+ cm (dark red, for tall plants). We trialled them in our paddy rice experiments and encountered segmentation issues as a consequence of unclear plot boundaries, changing water levels and overlapped rice plants during ripening (Notes S5).

Consequently, we developed an optimized plot segmentation algorithm (Notes S6): (1) applied an iterative self-organizing data thresholding (Irvin et al., 1997) to a field-level CHM and generated a global mask to represent plot edges (Fig. 2g, lower); (2) the Hough transform (Duda et al., 1972) was employed to seek horizontal and vertical lines in the mask, respectively (Fig. 2h, upper); (3) when some boundaries were undetectable, vertical and horizontal lines could be drawn manually to improve plot delineation via the GUI; (4) as most of the plots were not distanced evenly even with RTK-assisted seed drilling, we merged the adjacent lines; (5) after detecting plot boundaries, we assembled the remained lines to generate plot masks (Fig. 2h, lower), based on which all the plots were labelled according to the trial design for indexing and cross-referencing purposes (Fig. 2i, upper left); (6) to minimize edge effects and remove overlapped plants between neighbouring plots, a scaling function was designed to rescale the plot masks to measure different traits (e.g. scale = 0.25–0.3 for height measurements, depending on the degree of plant overlapping; Fig. 2i, upper right); and (7) finally, the refined masks were used to generate plot-level sampling regions (Fig. 2i, lower).

Automated trait analysis

Rice growth and development can be associated with stem elongation (i.e. changes in height) over time (Hosoi & Omasa, 2012). We utilized both spatial and spectral signals to analyze growth-related traits. For different morphological traits, varied vertical levels of spatial signals were used. For example, we chose the top 10% height values ($H_{90th}$, i.e. top 10% of the 3D points; see reasoning in Notes S7) in the CHMs (scale = 0.25–0.3) to compute canopy plant height after grain-filling. For early establishment, as colour or textural signals were unreliable in identifying seedlings owing to weedy plants and changing water levels, we therefore first segment plant signals from CHMs (scale = 0.9; Fig. 3a); then, seedling masks were generated using $H_{50th}$ as a result of short seedlings (Fig. 3b, left); after removing noisy objects (e.g. nongreen pixels) based on ExG values, we separated seedling objects from
their surrounding pixels using morphological erosion (Fig. 3b, middle), followed by indexing seedlings (Fig. 3b, right; Fig. S2).

Comparably, we applied similar steps to measure canopy coverage before canopy closure: (1) using $H_{50\text{th}}$ to represent plot canopy as canopy density was low during early vegetative phase (Fig. 3c, left); (2) applying the local adaptive thresholding (Singh et al., 2012) to generate a field-level mask (Fig. 3c, right); (3) overlapping the mask with 2D orthomosaic (Fig. 3d, left); (4) removing plot edges as some gaps between plots were unclear at canopy closure (scale $= 0.7$; Fig. 3d, middle); (5) using the Lab colour space (McLaren, 1976) to filter nongreen pixels (Fig. 3d, right); and (6) computing normalized canopy coverage index (CCI; 0 to 1, where 1 stands for 100% coverage; Notes S8).

According to a recent report (Svensgaard et al., 2021), RGB sensors can be applied to perform reliable spectral analysis without radiation calibration. Hence, we used RGB sensor to compute growth-related vegetation indices in the study. A series of vegetative indices and textural traits (e.g. canopy uniformity) were produced (Notes S9). All of the traits produced by AirMea\-surer are listed in Table 1.

Analysis of dynamic traits

Because dynamic or longitudinal phenotypes can be more informative in revealing plant–environment interactions (Campbell et al., 2019), we derived dynamic traits from static traits collected at different growth stages, rather than using values scored at arbitrary time points to represent growth patterns. Inspired by previous research (Anderson et al., 2019), we chose to measure dynamic phenotypes from the fitted curves even if some phenotyping points might be missing. The following section describes steps to compute dynamic phenotypes for an example trait, canopy height growth:

(1) Eight height values were used between sowing and grain-filling for a given *japonica* landrace (red dots in Fig. 4a). The eight points were relatively evenly distanced between 10 and 115 d after sowing (DAS). Because the height of rice canopy tends to decrease during the later grain-filling period, we tested several fitting functions (e.g. stepwise regression) and chose the Gaussian function to fit plant height changes (green curve, Fig. 4a).
The Gaussian-fitted height curve $f_x(\text{height})$ was then used to generate a growth-difference curve $f_x(\text{diff})$ (black dash curve, Fig. 4a) through the KneedLocator function (Satopää et al., 2011), which measures value changes on $f_x(\text{height})$, signifying the rate of plant height changes (i.e. increasing, decreasing or constant).

Turning points (i.e. knee points, KPs; red crosses, Fig. 4a) were located on $f_x(\text{diff})$, indicating height change phases. To locate these KPs, we found the first ($f_0 x(x)$) and second ($f_00 x(x)$) derivatives on $f_x(\text{diff})$; KP1 was detected when $f_0 x(x) = 0$ and $f_00 x(x) > 0$, whereas KP2 was detected when $f_0 x(x) = 0$ and $f_00 x(x) < 0$. We named the phase between KP1 and KP2 as rapid height growth phase ($RGP_{\text{height}}$; in days), denoting the period of rapid stem elongation.

Within the $RGP_{\text{height}}$, we found the first derivative $f'(x)_{\text{height}}$ (green curve) to locate the day when canopy height was changing at the fastest growth rate (i.e. the $FGR_{\text{height}}$ day, in DAS; light-green cross; Fig. 4a) together with computing average growth rate ($AGR_{\text{height}}$; %), between 0 DAS and the $FGR_{\text{height}}$ day.

Then, we applied the above steps to analyze indica and intermediate landraces (e.g. GP014 and GP543; Fig. 4b). $RGP_{\text{height}}$ values for the genotypes were identified together with the $FGR_{\text{height}}$ days and $AGR_{\text{height}}$. To assess phenotypic changes for other growth-related traits, we employed the algorithm to study variables such as ExG (i.e. $RGP_{\text{ExG}}, FGR_{\text{ExG}}$ and $AGR_{\text{ExG}}$) and CCI (i.e. $RGP_{\text{CCI}}, FGR_{\text{CCI}}$ and $AGR_{\text{CCI}}$). We also applied $f(x)_{\text{height}}$ green curves, Fig. 4c) to estimate $Max_{\text{height}}$ and other key growth stages (e.g. the beginning of ripening) using a normalized-curvature curve $f(x)_{\text{cuv}}$ (dotted blue, Fig. 4c). The maximum curvature on $f(x)_{\text{cuv}}$ was located to represent the $Max_{\text{height}}$ day (blue crosses, Fig. 4c), followed by the estimation of the beginning of ripening (purple crosses, Fig. 4c) using the minimum curvature. Moreover, $AGR_{\text{height}}, AGR_{\text{ExG}}$ and $AGR_{\text{CCI}}$ (all in %) between 0 DAS and the $Max_{\text{height}}$ day also were quantified. To compute $f(x)_{\text{cuv}}$, we used the equation below:

$$K = \frac{|f''(x)_{\text{height}}|}{\left(1 + \left(f'(x)_{\text{height}}\right)^2\right)^\frac{3}{2}}$$  
Eqn 1
Table 1 All of the traits that can be produced by the AirMeasurer platform (their equations, normalization, references and biological relevance are provided in Supporting Information Notes S9).

<table>
<thead>
<tr>
<th>Signals</th>
<th>Name (plot-based static traits)</th>
<th>Dynamic traits (based on static traits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological traits</td>
<td>1. Canopy plant height(^a) (all growth stages)</td>
<td>18. Rapid growth phase(^b) (RGP), for traits measured from 0 DAS to ripening.</td>
</tr>
<tr>
<td></td>
<td>2. Canopy coverage index(^a) (CCI), from 0 d after sowing (DAS) to canopy closure</td>
<td></td>
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<td></td>
<td>3. Seedling number(^b) (early establishment)</td>
<td></td>
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<tr>
<td>Spectral traits</td>
<td>6. Excess green(^a) (ExG; all stages)</td>
<td>19. Fastest growth rate(^b) (FGR), within the RGP of traits measured.</td>
</tr>
<tr>
<td>Textural traits</td>
<td>12. Greyscale co-occurrence matrices(^a) (GLCMs; stem elongation – ripening)</td>
<td>20. Average growth rate(^b) (AGR), the duration is changeable (e.g. 0 DAS – (Max_{\text{height}}) day, 0 DAS – the FGR day, or within the RGP).</td>
</tr>
</tbody>
</table>

\(^a\)Integrated in the GUI software. 
\(^b\)Executed via modules or trained models through Jupyter notebooks or Python scripts.

\(f(x_i)_{\text{height}}\), Gaussian-fitted height curve; \(i\) is between 10 and 115 DAS.

**Python-based software implementation**

A relative lack of open analytic solutions impedes researchers from exploiting newly introduced methods. Hence, we chose to develop the AirMeasurer GUI using Python programming language together with a modular design, so that each function or module in the platform could be accessed and modified independently. We used the Tkinter toolkit (Shipman, 2013) to develop a cross-platform GUI (in EXE). Open-source libraries such as SciPy, OpenCV and SciKit-Learn were employed to develop 2D/3D trait analysis algorithms and machine-learning based predictive modelling. The GUI software, executable Jupyter notebooks, and user guides are provided for academic use (Availability and Requirements).

**GWAS analysis and QTL mapping**

The AirMeasurer-measured and manual-scored traits collected from landraces were used to perform GWAS analysis to find the associated-loci controlling phenotypes. The RIL population was used to verify the static and dynamic traits through QTL mapping. For GWAS analysis, an efficient mixed-model association eXpedited (EMMEx) was performed (Kang et al., 2010). Single-nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) < 0.05 were excluded. Lines with missing phenotypes as a result of agronomic reasons were excluded. In total 254 landraces (2019/2020 seasons) were used to conduct GWAS. The matrix of pairwise genetic distances obtained by the simple SNP matching coefficients was employed to model the variance–covariance matrix of the random effect. Permutation tests were applied to help define the threshold of association signals (Churchill & Doerge, 1994). For each trait, we reshuffled the phenotypic data and performed association analysis using EMMEx with same parameters. To determine the significant threshold in GWAS, we accomplished 100 permutation analyses for each trait. Manhattan and quantile-quantile (QQ) plots were produced by using the Perl scripts (Mägi & Morris, 2010). For the 191 homozygous RILs (2020/2021 seasons), sequencing and genotyping were conducted using the published pipeline and SEG-MAP (Zhao et al., 2010). Windows QTL Cartographer v.2.5 (Wang, 2007) was employed for QTL analysis of composite interval mapping. LOD value was computed to indicate the possibility of QTLs based on likelihood ratio tests.

**Results**

Collected 2D/3D aerial images

Using low-cost UAVs to monitor rice experiments between 2019 and 2021, many series of 2D/3D aerial images were generated. For example, eight flights conducted in the 0.1-ha trial in Shanghai generated 10 GB 2D/3D imagery from over 100 GB raw images in a season. For the 0.2-ha trial in Hainan, 13 GB 2D/3D imagery was created from eight flights (145 GB raw images).
We uploaded a series of testing files to our GitHub repository (10 GB in total) for researchers to test and improve AIRMEASURER.

The GUI software

The initial GUI window of AIRMEASURER software consists of an input (red dash rectangle) section and a unified workspace (green dash rectangle; Fig. 5a). Users can select 2D/3D image series and a SHP file to begin the processing, including: (1) ‘tab a’ shows the central portion of the input orthomosaics within several seconds so that users can choose one image to proceed (Fig. 5b); (2) ‘tab b’ defines ROI and aligns the selected orthomosaic (Fig. 5c); (3) ‘tab c’ generates a CHM using the associated 3D point clouds and performs initial plot segmentation (Fig. 5d); (4) if some plot boundaries fail to delineate, users can use a mouse to draw horizontal and vertical lines to improve the plot delineation (yellow circles; Fig. 5e); (5) users can refine the plot masks using the scale function (0–1, where 1 stands for 100% of the original masks; Fig. 5d right); and (6) ‘tab d’ visualizes initial results and a button for batch processing with a progress bar and a checkbox for generating a performance matrix for genotypes (Fig. 5f). A different image can be selected in ‘tab a’ to repeat the above procedure. The initial plot masks will be used to benchmark all the input images during batch processing. Finally, plot-based trait analysis and processed plot-level images can be downloaded. Using an ordinary computer (Intel Core i7 CPU, 16 GB RAM with integrated graphics), 16 2D/3D images (10 GB) took 3 h to process. A detailed step-by-step user guide is provided in Notes S10 and Video S1.

Multiseason plant height analysis

We applied the AIRMEASURER system to process flights conducted in multiseason rice trials. For visual display, we selected three 2D orthomosaics to present overhead imagery and three 3D point clouds to exhibit field-level plant spatial features, from a 30° perspective, on 53, 73 and 103 DAS in the 2019 season, when landraces entered vegetative, reproductive and ripening phases (Fig. 6a–c, left). Pseudocoloured height maps (Fig. 6a–c, right; with a unified scale bar) were created using AIRMEASURER-derived...
height measures, showing height changes of the 254 landraces during the season. We applied the Gaussian-fitted curves and categorized the landraces into three groups according to their domestic types (i.e. *indica*, *japonica* and intermediary), with coloured shading areas denoting 15th–85th percentile confidence intervals (Fig. 6d).

The fitted curves roughly followed a sigmoid pattern but with dissimilar developmental rates. For example, the intermediary group peaked on 91 DAS ($\text{Max}_{\text{height}} = 1.18$ m), followed by the *japonica* group (93 DAS; $\text{Max}_{\text{height}} = 0.97$ m) and the *indica* group (94 DAS; $\text{Max}_{\text{height}} = 0.82$ m). For the 2020 height measures, three flights (on 52, 70 and 100 DAS) were selected to visualize plot-based height differences (Fig. 6e–g). The Gaussian-fitted curves identified similar growth patterns (Fig. 6h); for example, the $\text{Max}_{\text{height}}$ days are between 95 and 110 DAS, and the 2020 lines were 5–10 cm shorter than the same lines studied in 2019. Complete height measurements for the two seasons (Datasets S1, S2), 2D orthomosaics and pseudocoloured height maps for the 191 RILs are provided (Fig. S3).

Performance matrix and dynamic trait analysis

In order to analyze dynamic traits effectively, we created a new function to help reorganize plant genotypes. For example, by extracting plot-level images from the 254 rice landraces and inserting them into a matrix according to their domestic groups, a ‘performance matrix’ was created using eight orthomosaics collected between 20 July and 8 October 2019 (Fig. 7a). In the matrix, each cell was an overhead image of a rice genotype, such that genotypes were columns and phenotyping timepoints were rows (see the entire 2019 performance matrix in Notes S11).

We used the matrix to examine different traits. The first row was utilized to quantify plot-based seedling number (Dataset S3; Fig. S2). To perform dynamic analysis of traits such as CCI, we used Gaussian-fitted curves to study the increase of CCI until 100% coverage was reached (Fig. 7b), based on which $\text{FGR}_{\text{CCI}}$, $\text{AGR}_{\text{CCI}}$ and the $\text{Max}_{\text{CCI}}$ day were computed (Dataset S4). For spectral traits such as ExG and visible atmospherically resistant index (VARI), their associated dynamic traits were also quantified.
Likewise, the matrix was employed to estimate dynamic changes for other indices (Dataset S7; Fig. S4). Noticeably, to estimate height-related traits (e.g. AGR$_{\text{height}}$, RGP$_{\text{height}}$ and FGR$_{\text{height}}$; Dataset S9), both the matrix and associated CHMs were used.

Estimation of heading date

Based on dynamic traits, we explored the estimation of a complex trait, heading date. We predicted the trait using multiple dynamic traits (e.g. height, CCI and VARI) and machine-learning modelling (Notes S12), including: trait selection (Fig. S5a), dynamic trait analysis (Fig. S5b), feature engineering and selection (Fig. S5c), model training and selection (Fig. S5d), and model validation (Fig. S5e). Among many models tested, support vector regression (SVR) obtained the best coefficient of determination ($R^2 = 0.725$; with $P < 0.001$ in linear regression; Notes S12).

Validation of AIRMEASURER-derived traits

AIRMEASURER-derived traits were validated by a range of ground truth data. The correlation between the 2019-AIRMEASURER-derived $Max_{\text{height}}$ and the 2019-manual-scored $Max_{\text{height}}$ was visualized using 177 landraces ($R^2 = 0.8848$, $P < 0.001$; root mean square error, RMSE = 16.041; Fig. 8a), showing a very strong positive correlation. We repeated the validation with 254 landraces measured in the field in 2020. A similar $R^2$ was obtained (0.8926, $P < 0.001$, RMSE = 21.163; Fig. 8b). Owing to different methods, AIRMEASURER-derived height values were consistently shorter than manual scoring, which was expected as plants were straightened in manual scoring. The result indicates that AIRMEASURER could soundly estimate maximum plant height among diverse rice genotypes (including landraces) with a high accuracy. We also verified the AIRMEASURER-measured height at eight time points (35–115 DAS) using 1416 plots (177 plots per time point) against plot-based canopy height that was measured manually from eight 3D point clouds ($R^2 = 0.9651$, $P < 0.001$, RMSE = 6.675; Fig. 8c), as well as against the Gaussian-fitted canopy height ($n = 1416$ plots; $R^2 = 0.9649$, $P < 0.001$, RMSE = 6.092; Fig. 8d). Significant positive correlations were obtained, indicating the reliability of the AIRMEASURER-estimated height trait throughout the season. For traits such as ExG and CCI that also were used for genetic mapping, 177 plots (at six growth stages, with 29–30 plots per stage) were used to compare the two traits obtained by manual and AIRMEASURER-based
approaches, both resulting in strong correlations: \( R^2 = 0.9497 \) for CCI (\( P < 0.01; \) Fig. 8e) and \( R^2 = 0.9091 \) for ExG (\( P < 0.001; \) Fig. 8f).

QTL mapping using height-related traits

In order to evaluate the biological relevance of AirMeasurer-derived traits in genetic mapping studies, we first used the overlapped 191 homozygous RILs in 2020 and 2021 season for genetic linkage analysis. The AIRMEASURER-derived \( \text{Maxheight} \) trait was used to map QTLs in the population, with the \( x \)-axis representing the genetic distance of 12 chromosomes and \( y \)-axis the LOD value. The threshold (red horizontal line) was set as 2.5 and known loci were indicated with red arrows. Two QTLs related to \( \text{Maxheight} \) were identified (Fig. 9a), among which one significant QTL (LOD = 15.7; chromosome 1) indicated a locus controlling rice plant height in the two seasons, consistent with the QTL mapping using the 2021 manual data and reported previously using the same population (Wang et al., 2011). In fact, there is a known gene \( sd1 \) that shortens rice stems (Sasaki et al., 2002), c. 100–210 kb from the locus. The second highest peak located using the \( \text{Maxheight} \) trait was on chromosome 7 (LOD = 7.3), c. 0 kb from \( Ghd7.1 \), a gene plays an important role in grain productivity and rice heading (Yan et al., 2013); however, the second peak identified with the manual scoring was c. 1 Mb from \( Ghd7.1 \) (LOD = 7.9; Fig. 9b).

Besides the static trait, we applied the dynamic trait, \( \text{AGRheight} \), to map QTLs and several QTLs were detected. Using the 2020 \( \text{AGRheight} \) (0 DAS – the \( \text{Maxheight} \) day), two QTLs were identified (Fig. 9c): c. 110 kb from \( sd1 \) (LOD = 18.6) and c. 0.97 mb from \( Ghd7.1 \) (LOD = 6.5). The 2021 \( \text{AGRheight} \) (0 DAS – the \( \text{FGRCCI} \) day) was used and located same QTLs (Fig. 9d): c. 100 kb from \( sd1 \) (LOD = 9.3) and c. 30 kb from \( Ghd7.1 \) (LOD = 3.2). In particular, we identified four loci with the 2020 \( \text{RGPheight} \) trait (Fig. 9e), two strong signals are: (1) c. 250 kb from \( SUJ2 \) (LOD = 4.9), which regulates rice stem development (Virlet et al., 2017); (2) c. 3.7 Mb on chromosome 12 (LOD = 7.4), which is not associated with any known gene.

QTL mapping using growth-related traits

Then, we mapped QTLs using other AIRMEASURER-derived growth traits such as CCI and ExG. The 2021 \( \text{AGRCCI} \) (0 DAS – the \( \text{FGRCCI} \) day) was used to locate two QTLs (Fig. 9f), including \( Osbox4 \) (c. 0 kb; LOD = 7.9), overexpressing the gene leads to dwarfing and increased tillers, and thus the canopy size (Dai et al., 2008). One strong locus was identified on chromosome 9 (LOD = 9.6; Fig. 9g) using the 2021 \( \text{AGRCCI} \) trait (0 DAS – the \( \text{MaxCCI} \) day), indicating a locus linking to canopy expansion. Actually, \( TAC1 \) (Yu et al., 2007) is c. 10 kb away, which controls a spread-out or compact plant architecture. The
QTL (19.2–21.6 Mb) had three peaks: 20.05 Mb, 21.05 Mb and 21.55 Mb, respectively (detailed in Fig. 9g); besides TAC1, there is a gene at 20.07 Mb, LGD1 (Thangasamy et al., 2012), which regulates vegetative growth. By mapping QTLs using the 2020 AGRExG (0 DAS–MaxExG day), several vegetation-related QTLs were identified on chromosome 1 (LOD = 6.1; Fig. 9h), including: (1) SLB1 and SLB2 (Cardoso et al., 2014), controlling tillering, and (2) D61 (Yamamuro et al., 2000), connected with internode elongation. All of the QTLs identified through QTL mapping are listed in Table 2.

Fig. 9 Genetic linkage analysis of various AIRMEASURER-derived growth-related traits and manually scored maximum plant height, collected from 191 homozygous recombinant inbred lines (RILs) trialled in 2020 and 2021. For the significant single-nucleotide polymorphisms (SNPs) identified, known genes are indicated by red arrows. (a) Chromosomal location of significant quantitative trait locus (QTLs) identified using AIRMEASURER-derived Maxheight trait in 2020. The x-axis denotes the genetic distance of 12 chromosomes and y-axis the logarithm of the odds (LOD) value, with a significant threshold set at 2.5 (red horizontal line). The QTLs are close to the sd1 gene (chromosome 1) and the Ghd7.1 gene (chromosome 7). (b) Height QTLs identified using manually measured maximum plant height in the 2021 season; these also were located close to the sd1 and Ghd7.1 genes. (c) QTL for the AGRheight trait, between 0 d after sowing (DAS) and the Maxheight day, in 2020. (d) QTL for the AGRheight trait (0 DAS – the FGRheight day) in 2021. (e) Four loci associated with the RGPheight trait collected in the 2020 season, including one located near SUI2 (chromosome 5), and another significant locus on chromosome 12 that is not associated with any known gene. (f) Two QTLs for the AGRCCI in 2021, determined over the period between 0 DAS and the FGRCCI day. The major QTL co-locates with Oshox4. (g) QTL for the average growth rate of CCI in 2021 determined over the period between 0 DAS and the MaxCCI day. One strong loci on chromosome 9 (three peaks between 19.2 Mb and 21.6 Mb) co-locates with a known gene (TAC1) that controls canopy structure, and LGD1 that regulates vegetative growth in rice. (h) QTLs for the AGRCCI trait for the interval 0 DAS–MaxCCI day. The major QTL co-locates with SLB1/SLB2 and D61. Table 2 summarizes the QTLs associated with the above growth-related traits. Abbreviations: maximum canopy height (Maxheight; cm), average growth rate for a target trait (AGRtrait; %), the fastest growth rate of canopy height (FGRheight; %), the rapid growth phase (RGPheight; days), canopy coverage index (CCI), excess green (ExG), maximum CCI (MaxCCI), maximum ExG (MaxExG) and the fastest growth rate of CCI (FGRCCI; %).
GWAS using height-related traits

Besides QTL mapping, we utilized AirMEASURER-derived traits in GWAS analysis with the 254 rice landraces. We identified several significant SNPs associated with the two-season height-related traits and presented them in the Manhattan plot and QQ plot, with a grey dotted line indicating the threshold of the genome-wide significant P-value (Table S1) and a false detection...
rate (FDR) of 0.2. For example, using the 2019 Max_height trait, the strongest signal on chromosome 1 (−log10(P) = 12, indicated with a blue arrow; Fig. 10a, left) was c. 208 kb from sd1. On chromosome 3, the strongest signal (−log10(P) = 6.42) identified was c. 10.7 kb from the OsHox32 gene, which is known for pleiotropic effects on plant architecture and leaf development (Chen et al., 2021). We repeated the analysis using the 2020 Max_height trait and produced similar results on chromosome 1 (c. 208 kb from sd1; −log10(P) = 7.14; Fig. 10a, right). The findings were consistent with the GWAS analysis using the two-season manual Max_height scoring (c. 202–208 kb from sd1; −log10(P) = 6.91–6.63; Fig. 10b).

Next, we chose the 2019 AGR_height (0 DAS – the Max_height day) and identified four significant SNPs associated with the trait (Fig. 10c, left). Besides the strongest signal (−log10(P) = 10.62; c. 208 kb from sd1), the second strongest signal (−log10(P) = 6.98) was c. 11 kb from OsHox32 on chromosome 3, followed by a strong signal (−log10(P) = 6.6) c. 122 kb from NOG1 on chromosome 1, and the last one c. 373 kb from OsGSK2 on chromosome 5. The NOG1 gene increases rice grain production (Huo et al., 2017), whereas OsGSK2 regulates the mesocotyl length (Sun et al., 2018), both genes relate to plant growth and development. We repeated the analysis using the 2020 data (Fig. 10c, right) and reproduced two SNPs that close to sd1 (−log10(P) = 8.29; c. 188 kb) and OsGSK2 (−log10(P) = 8.07; c. 373 kb). To test other height-related dynamic traits, we used the two-season AGR_height (0 DAS – the FGR_height day) and obtained similar results (Fig. 10d), with a significant SNP on chromosome 5 repeatedly located (−log10(P) = 8.28–10.28; c. 306.1–372.98 kb away from OsGSK2). Table 3 lists all the significant signals associated with the above traits.

GWAS using other growth-related traits

Finally, we chose the AirMeasurer-derived CCI and ExG traits to perform GWAS and found three SNPs (Table S2). Using the 2020 AGR_CCI trait (0 DAS – the Max_CCI day) two signals were identified (Fig. 10e): (1) one signal (−log10(P) = 7.4) was c. 329 kb from the Pit gene on chromosome 1, a disease resistance gene (Hayashi & Yoshida, 2009); (2) another (−log10(P) = 6.49) was c. 224 kb from the PPFP gene on chromosome 5, which associates with carbon metabolism during grain-filling (Duan et al., 2016). Using the 2019 AGR_Exp trait (0 DAS – the FGR_Exp day), the strongest signal (−log10(P) = 6.07) was c. 304 kb from the CCP1 gene on chromosome 1 (Fig. 10f), which functions palea development.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Year</th>
<th>chr.</th>
<th>Peak gent. pos.</th>
<th>IRGSP4.0 (Mb)</th>
<th>LOD</th>
<th>R²</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max_height (cm), AirMeasurer</td>
<td>2020</td>
<td>1</td>
<td>374.0</td>
<td>40.25</td>
<td>12.8</td>
<td>22.3%</td>
<td>sd1 (40.14 Mb)</td>
</tr>
<tr>
<td>Max_height (cm), AirMeasurer</td>
<td>2020</td>
<td>7</td>
<td>195.3</td>
<td>30.20</td>
<td>9.4</td>
<td>15.4%</td>
<td>Ghd7.1 (30.28 Mb)</td>
</tr>
<tr>
<td>Max_height (cm), AirMeasurer</td>
<td>2021</td>
<td>12</td>
<td>195.7</td>
<td>22.05</td>
<td>4.0</td>
<td>5.9%</td>
<td>Ghd7.1 (30.28 Mb)</td>
</tr>
<tr>
<td>Maximum plant height (cm), manual</td>
<td>2021</td>
<td>1</td>
<td>374.3</td>
<td>40.35</td>
<td>15.9</td>
<td>24.2%</td>
<td>sd1 (40.14 Mb)</td>
</tr>
<tr>
<td>Maximum plant height (cm), manual</td>
<td>2021</td>
<td>7</td>
<td>187.7</td>
<td>29.25</td>
<td>7.9</td>
<td>10.7%</td>
<td>Ghd7.1 (30.28 Mb)</td>
</tr>
<tr>
<td>Maximum plant height (cm), manual</td>
<td>2021</td>
<td>12</td>
<td>195.7</td>
<td>22.05</td>
<td>3.3</td>
<td>4.3%</td>
<td>Ghd7.1 (30.28 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>1</td>
<td>374.0</td>
<td>40.25</td>
<td>18.6</td>
<td>30.0%</td>
<td>sd1 (40.14 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>7</td>
<td>188.7</td>
<td>29.30</td>
<td>6.5</td>
<td>8.9%</td>
<td>Ghd7.1 (30.27 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>12</td>
<td>162.6</td>
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<td>3.5</td>
<td>4.6%</td>
<td>Xa25/Os12g0476200 (17.4 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2021</td>
<td>1</td>
<td>374.0</td>
<td>40.25</td>
<td>9.3</td>
<td>16.8%</td>
<td>sd1 (40.14 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2021</td>
<td>7</td>
<td>196.3</td>
<td>30.30</td>
<td>3.2</td>
<td>5.3%</td>
<td>Ghd7.1 (30.27 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2021</td>
<td>12</td>
<td>191.2</td>
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<td>3.5</td>
<td>5.8%</td>
<td>OsVIL2/Os12g0535300 (21.39 Mb)</td>
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<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>2</td>
<td>216.7</td>
<td>26.40</td>
<td>3.4</td>
<td>6.0%</td>
<td>OsYABBY4/Os02g0643200 (26.7 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>5</td>
<td>225.4</td>
<td>27.85</td>
<td>4.9</td>
<td>8.9%</td>
<td>SU2/Os05g0055400 (27.6 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2020</td>
<td>7</td>
<td>0.0</td>
<td>0.50</td>
<td>3.3</td>
<td>4.9%</td>
<td>OsGA2ox5/Os07g0103500 (0.2 Mb)</td>
</tr>
<tr>
<td>AGR_height (%; 0 DAS – the Max_height day), AirMeasurer</td>
<td>2021</td>
<td>12</td>
<td>49.9</td>
<td>3.70</td>
<td>7.4</td>
<td>14.6%</td>
<td>Unknown</td>
</tr>
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<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
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<td>1</td>
<td>301.7</td>
<td>30.60</td>
<td>6.1</td>
<td>12.4%</td>
<td>SLB1/0.377 Mb, SLB2/0.308 Mb, D61 (31.6 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
<td>2020</td>
<td>5</td>
<td>216.6</td>
<td>27.30</td>
<td>4.1</td>
<td>8.0%</td>
<td>SU2/Os05g0554000 (27.6 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
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<td>9</td>
<td>145.1</td>
<td>20.05</td>
<td>10.0</td>
<td>17.4%</td>
<td>LGD1/Os09g0502100 (20.07 Mb)</td>
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<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
<td>2021</td>
<td>9</td>
<td>149.5</td>
<td>20.95</td>
<td>9.7</td>
<td>16.9%</td>
<td>OsMADS5/0.09g0507200 (20.3 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
<td>2021</td>
<td>9</td>
<td>154.3</td>
<td>21.55</td>
<td>9.6</td>
<td>16.8%</td>
<td>TAC1 (21.56 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
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<td>11</td>
<td>72.2–72.9</td>
<td>6.4–6.8</td>
<td>3.1</td>
<td>4.8%</td>
<td>Pia/Os11g0225100 (6.5 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
<td>2021</td>
<td>3</td>
<td>264.0–264.4</td>
<td>31.35–31.6</td>
<td>3.5</td>
<td>6.1%</td>
<td>OsIAA13/Os03g0742900 (31.2 Mb)</td>
</tr>
<tr>
<td>AGR_Exp (%; 0 DAS – the Max_Exp day)</td>
<td>2021</td>
<td>9</td>
<td>131.7</td>
<td>18.55</td>
<td>7.9</td>
<td>14.5%</td>
<td>Os2HD1 (18.35 Mb), OsZIP73 (18.55 Mb), OsZIP73 (18.77 Mb)</td>
</tr>
</tbody>
</table>
Fig. 10 Manhattan plots and quantile-quantile (QQ) plots for AIRMEASURER-derived traits subjected to a genome-wide association study (GWAS) of 254 rice landraces trialled in 2019 and 2020. The significance threshold is shown by the horizontal grey dotted line. Known genes that co-locate with significant loci are indicated by blue arrows. See Fig. 9 legend for trait abbreviations. (a) Manhattan plot and a QQ plot for the AIRMEASURER-derived Maxheight trait measured in 2019. The strongest signal on chromosome 1 was close to the sd1 gene and a strong signal on chromosome 3 was close to the OsHox32 gene. (b) Manhattan plot for the manually scored maximum plant height trait collected in the 2019 and 2020 seasons. (c) The 2019 AGRheight (0 DAS – the Maxheight day) was used to identify four significant SNPs, co-locating with known genes: sd1, OsHox32 (chromosome 3), NOG1 (chromosome 1) and OsGSK2 (chromosome 5). Analysis repeated using the same trait collected in 2020 and reproduced two SNPs, close to sd1 and OsGSK2. (d) GWAS performed with the trait AGRheight (0 DAS – the FGRheight day). Similar results were obtained in both seasons. (e) Plots for the 2020 AGRCCI (0 DAS – the MaxCCI day) trait. Two signals were identified, one close to the Pit gene on chromosome 1 and the other near the PFPβ gene on chromosome 6. (f) In the analysis of the 2019 AGRExG trait (0 DAS – the FGRExG day), the strongest signal co-located with the CCP1 gene on chromosome 1. Table 3 lists all of the significant association signals of the above growth-related traits. DAS, days after sowing.
Furthermore, GWAS was attempted with the 2019-heading-date trait estimated by both manual and AIRMEASURE approaches (Notes S13).

**Discussion**

In order to exploit available genomic resources to address climate change challenges, selected traits need to be assessed under field conditions across locations and years. Conventional phenotyping requires making many measurements of target traits, which is arduous and difficult to implement at busy periods of the season, resulting in newly developed methods (Pieruschka & Schurr, 2019; Jang et al., 2020). This study demonstrates that the use of low-cost UAVs can acquire larger and regular plant data from the field, based on which high-quality 2D/3D aerial imagery with field- and plot-level resolutions can be generated to enable automated analysis of static and dynamic traits that are biologically relevant. Furthermore, this approach is potentially valuable for assessing rates of genetic gain in larger trials, facilitating the calculation of heritability for agronomic traits and accurate genetic mapping for developing molecular markers. Nevertheless, metrics such as economic costs, scalability, analysis accuracy and throughput, or processing time need to be considered to evaluate the above research objectives, which is beyond the scope of this study but important for future studies.

Table 3 Genome-wide significant association signals of height-related traits collected from 254 rice landraces using EMMAx.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Yr.</th>
<th>Chr.</th>
<th>Positiona</th>
<th>(-\log_{10}P)</th>
<th>Distanceb (kb)</th>
<th>Candidate genes</th>
<th>Gene symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maxheight (cm), AirMeasurer</strong></td>
<td>19</td>
<td>1</td>
<td>40 349 753</td>
<td>12</td>
<td>208.421</td>
<td>Os01g0883800</td>
<td>sd1</td>
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<tr>
<td></td>
<td>3</td>
<td>25 428 611</td>
<td>6.42</td>
<td>10.659</td>
<td>Os03g0640800</td>
<td>OsHox32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1</td>
<td>40 349 753</td>
<td>7.14</td>
<td>208.421</td>
<td>Os01g0883800</td>
<td>sd1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2178 057</td>
<td>7.03</td>
<td></td>
<td></td>
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<td></td>
<td>9</td>
<td>15 190 326</td>
<td>7.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Maximum plant height (cm), manual</strong></td>
<td>19</td>
<td>1</td>
<td>33 179 314</td>
<td>9</td>
<td>202.126</td>
<td>Os01g0883800</td>
<td>sd1</td>
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<td><strong>AGRheight (%) 0 DAS – the</strong></td>
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<td>1</td>
<td>33 186 547</td>
<td>6.6</td>
<td>122.199</td>
<td>Os01g0752200</td>
<td>NOG1</td>
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<td><strong>MaxHeight day), AirMeasurer</strong></td>
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<td>25 429 147</td>
<td>6.98</td>
<td>11.195</td>
<td>Os03g0640800</td>
<td>OsHox32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6266 446</td>
<td>6.09</td>
<td>373.094</td>
<td>Os05g0207500</td>
<td>OsGSK2</td>
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<td><strong>AGRheight (%) 0 DAS – the</strong></td>
<td>20</td>
<td>1</td>
<td>40 329 637</td>
<td>8.29</td>
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\(^a\)Position in bp according to IRGSP 4.0.

\(^b\)The distance between SNP and candidate gene. DAS, d after sowing.

(Yan et al., 2015). The use of AIRMEASURER helped us analyze target traits coherently, which was achieved by removing unwanted field-level terrain features, transferring 3D to 2D signals for efficient processing, analyzing 3D points at different vertical levels and identifying plots consistently. These methodological advances were proved to be useful in examining static traits (e.g., height, CCI and ExG) at different growth stages for paddy rice (in particular the landraces), which was complex to study during the season.

Inspired by previous work (Würschum et al., 2014; Anderson et al., 2019), we developed a bespoke approach to estimate dynamic traits derived from time series measures of static traits, enabling us to gain insights into dynamic features (e.g. growth rate and RGP) of target traits, without excessive phenotyping. Instead of using phenotypes measured at arbitrary time points, dynamic analysis helped us evaluate phenotypic variation reliably with hundreds of genotypes.

Additionally, through integrating dynamic traits into machine-learning modelling, we predicted a complex trait, heading date, which could lead to new estimates of QTL x Environment interactions. Recent studies (Lowry et al., 2019; Mu et al., 2022) have reported similar approaches that used multi-location traits in QTL mapping. Also, we demonstrated Static and dynamic traits

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that AirMeasurer-derived traits could be used for multiseason QTL discovery, which was confirmed by the results highlighting the locations of known QTLs. Although the main objective of this study was not to discover novel QTLs, nor to validate robustness of such QTLs across different germplasm sets and environments, it would only require simple adjustments to trial designs (e.g. more replicates) and greater repetition of trials across locations and years in order to produce reliable estimates of trait heritability and QTLs.

**AirMeasurer as a research tool**

Genetic mapping of dynamic or longitudinal traits can be a powerful tool for developing novel molecular markers that cannot easily be revealed using static measurement, partly because of temporal regulation of gene expression (Harder et al., 2019). We explored the use of AirMeasurer-derived traits to identify associated loci. For example, QTLs were mapped for traits such as Max$_{Height}$, AGR$_{beige}$ during RGP$_{beige}$ or at the FGR$_{beige}$ day. If the QTLs are shown to be robust across years, locations and different germplasm sets, these could then be used to develop growth-related molecular markers. Some of the identified loci were co-located with known genes, as well as with other genes within the interval had unknown functions, which could lead to new candidate genes. QTLs located using traits derived from CCI and ExG (e.g. Max$_{conv}$, FGR$_{conv}$ and Max$_{ECG}$) also indicate potentially useful loci.

Likewise, we used AirMeasurer-derived traits in GWAS analysis. Comparable loci were identified from rice landraces. Height-related trait (e.g. AGR$_{beige}$) led to the consistent identification of signals such as the nearby genes (e.g. sd1, OsHox32, NOG1 and OsGSK2) relevant to plant height, architecture and growth regulation, indicating the value of dynamic traits in studying genetically diverse landrace populations. Moreover, using dynamic traits such as AGR$_{beige}$, Max$_{conv}$ and FGR$_{ECG}$, we located some previously unknown strong signals, which may be valuable for identifying small effects of individual allelic differences (e.g. loci on Chromosome 2; Fig. 10c,d) that jointly contribute to the regulation of trait expressions. Finally, AirMeasurer-estimated heading date traits could bring a new perspective to GWAS analysis. Loci identified using a small number of indica landraces ($n = 97$) were just $c. 17.14$ kb from OsSOC1 gene on chromosome 3 ($-\log_{10}(P) = 5.79$) and $c. 30.24$ kb from the Hd3a gene on chromosome 6 ($-\log_{10}(P) = 4.65$), both of which are heading-date related (Notes S13).

**Limitations of the platform**

We have encountered problems that are not uncommon when applying drones in aerial phenotyping: (1) weather conditions – small UAVs cannot be operated in unstable weather such as high or gusty wind ($> 15$ m s$^{-1}$), rainfall, or heavy fog (Tmušić et al., 2020); (2) geo-referencing – GPS modules installed on low-cost drones had metre-level deviation and thus geo-referencing errors needed to be rectified; (3) nature illuminance – image colour and contrast could vary noticeably with changing light conditions, and we mitigated this issue by conducting a field-level imaging; and (4) aviation regulations – the change in aviation regulations casts uncertainty on aerial phenotyping, requiring regular communications with local civil air traffic control authorities; without official authorization, the payload capacity of a drone was restricted, indicating the advantage and practicality in using small drones for routine phenotyping. In the study, we used an RGB camera for growth-related spectral analysis as a low-cost alternative to more costly hyper- or multispectral sensors. It is worth noting that visible spectra are limited in early diseases detection and sensing abiotic stress responses as accurate spectral information is key to assess plant responses to certain external stimuli (Tmušić et al., 2020).

For data pre-processing, we used the proprietary Pix4DMapper software to generate 3D point clouds and 2D orthomosaics. We have tested several open-source software types (e.g. VISUALSFM, Meshroom) for the same task and encountered technical problems such as prolonged computational time, incorrect geo-referencing, and mismatched 2D/3D patches. Another problem during the processing was to denoise large-scale 3D point clouds. We used the SQR for the task, which required 15–20 min to denoise 60+ million 3D points. Hence, algorithms such as local-outlier and cluster-based outlier detection (Kriegel et al., 2009), and deep-learning (DL) methods (Casajus et al., 2019) should be considered to speed up this task. Although the AirMeasurer’s plot segmentation algorithm could reliably be applied to field experiments with regular grided plot layout designs (Notes S10), it cannot be extended to analyze irregular plot layouts (e.g. zigzag arrangements). DL approaches such as multilayer perceptron that can incorporate multidimensional ground/plant signals might be more useful for this mission.

**Future applications**

Further developments could include the analysis of high-density 3D point clouds. Rice flowering starts 1 d after the heading, during which anthers (1–1.5 mm in diameter) can be observed on different panicles. By flying smaller UAVs (e.g. DJI Mini) at a 4-n altitude, we could achieve a ground-sampling-distance (GSD) of 1–1.5 mm per pixel. Thus, it is feasible to measure anther extrusion using high-density 3D points acquired by smaller drones, which could find applications in hybrid breeding programmes where the selection of male parents with certain flowering characteristics is crucial.

Low-cost UAVs and dynamic trait analysis also could be applied to examine traits such as grain-filling, which are challenging to quantify using conventional approaches. By conducting daily flights during ripening, fitted curves could enable the estimation and eventually the prediction of the initiation and duration of this key trait. However, it is expected that the Gaussian function might not be suitable for such growth patterns, and thus other fitting methods shall be explored.

Although we did not thoroughly test AirMeasurer to analyze other crops, we have successfully applied the platform to examine wheat trials with limited parametric changes (Notes S10, S14), suggesting potential applications of AirMeasurer for other plant species. As the modular-designed AirMeasurer was developed in Python, which is widely supported, we trust that this platform could be shared, extended and upgraded by the community relatively easily, providing open and readily accessible solutions for the broader research community.
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Competing interests

None declared.

Author contributions

Ji Zhou wrote the manuscript with inputs from EO, GS, QZ, YZ and HL; Ji Zhou, GS and Jie Zhou designed the AirMEASURER platform and GUI software with help from QZ and HL; GS, Jie Zhou, HL and Ji Zhou implemented the software; YW, AW, GS and LX performed the rice experiments and UAV-based field phenotyping under BH, QZ and Ji Zhou’s supervision; RJ and EO provided expertise in aerial imaging and crop modelling; GS, Jie Zhou and HL tested and optimised the software; Ji Zhou, GS, EO, JC and QZ performed the data analysis and modelling; and YZ, AW, HL, Ji Zhou and QZ performed GWAS analysis and QTL mapping under BH’s supervision. All authors read and approved the final manuscript. GS, HL and YZ contributed equally to this work.

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Open access

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ORCID

Joshua Colmer https://orcid.org/0000-0002-0511-685X
Bin Han https://orcid.org/0000-0001-8695-0274
Robert Jackson https://orcid.org/0000-0002-8364-1633
Hengyun Lu https://orcid.org/0000-0003-1166-6097
Eric Ober https://orcid.org/0000-0002-4832-5897
Gang Sun https://orcid.org/0000-0002-6548-5980
Ahong Wang https://orcid.org/0000-0003-1802-7562
Yongchun Wang https://orcid.org/0000-0003-1800-6446
Qiang Zhao https://orcid.org/0000-0002-8372-4463
Yan Zhao https://orcid.org/0000-0001-7721-3758
Ji Zhou https://orcid.org/0000-0002-5752-5524
Jie Zhou https://orcid.org/0000-0001-7162-4791

Data availability

Project name: AirMeasurer for genetic mapping in crops. Project release page and source code: https://github.com/The-Zhou-Lab/UAV/releases/tag/V2.0.2. Testing 2D/3D aerial images: 2D orthomosaics (x8) and 3D point clouds (x8), 11.3 GB in total. GUI software: AirMeasurer_v2.0.2.zip (480 MB). Operating system(s): Windows 10 onwards; the Jupyter notebook can be executed across platforms.

Requirements: Python 3.7+; laspy (1.7.0), Whitebox (1.3.0), GDAL (3.2.1), Rasterio (1.2.0), CSF (1.1.1), scikit-image (0.16.2), OpenCV-Contrib-Python (3.4.2.16), Pandas (1.0.1), NumPy (1.18.1), and SciPy (1.4.1).

References


Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 The 2019 season AIRMEASURER-measured height values.

Dataset S2 The 2020 season AIRMEASURER-measured height values.

Dataset S3 The 2019 season AIRMEASURER-estimated seedling number.

Dataset S4 The 2019 season dynamic analysis of canopy coverage index.

Dataset S5 The 2019 season dynamic analysis of the excess green index.

Dataset S6 The 2019 season AIRMEASURER-measured VARI index.

Dataset S7 The 2019 season AIRMEASURER-measured NDYI index.

Dataset S8 The 2019 season AIRMEASURER-measured ASM index.

Dataset S9 The 2019 season dynamic analysis of canopy height.

Fig. S1 Multilocation phenotyping using low-cost UAVs, customized flight plans and in-field setups.

Fig. S2 Plot-based seedling measurements to examine the number of seedlings for 241 RILs in January 2020 in the Hainan trial centre.

Fig. S3 Six overhead 2D orthomosaics, pseudocoloured height maps and 3D point clouds from 241 RILs, showing plant height changes between 15 January and 31 March 2020.

Fig. S4 Range of vegetative indices and textural traits measured by AIRMEASURER from 241 RILs between 15 January and 31 March 2020.

Fig. S5 Combining phenotypic traits and supervised machine learning to predict a complex trait, heading date, with high confidence.

Notes S1 Trial design and plant materials.

Notes S2 UAV imaging protocol and in-field setups.

Notes S3 Different plant height measures before and after removing terrain features.

Notes S4 3D point clouds processing and canopy height model.

Notes S5 Previous published segmentation solutions trialed in rice field experiments.

Notes S6 Source code of the plot segmentation algorithm.

Notes S7 The reasoning behind choosing $H_{90th}$ for height measurement.

Notes S8 Source code for computing canopy coverage and ExG indices.

Notes S9 Vegetative indices and texture-based traits.

Notes S10 A step-by-step user guide of the AIRMEASURER GUI.

Notes S11 Entire performance matrix for all plots monitored.

Notes S12 Estimation of a complex trait – heading date.

Notes S13 GWAS using heading dates estimated by the SVR model.

Notes S14 Applying AIRMEASURER to examine wheat plots under different nitrogen treatments.

Table S1 The genome-wide significant $P$-value (FDR0.2).

Table S2 Genome-wide significant association (GWAS) signals of ExG and CCI using EMMAx.

Video S1 GUI of AIRMEASURER in operation.

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