

RESEARCH ARTICLE

Towards increased shading capacity: A combined phenotypic and genetic analysis of rice shoot architecture

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Funding information

This research was funded by the Netherlands Organisation for Scientific Research (NWO) (Project Number 14700.RS) in collaboration with The International Rice Research Institute.

Societal Impact Statement

Rice farming is transitioning from transplanting rice seedlings towards the less labour-intensive and less water-demanding method of directly seeding rice. This, however, is accompanied by increased weed proliferation. To tackle this issue, this study seeks to identify how the crop itself can better suppress weeds, with a focus on light competition via shading. Using a rice diversity panel, traits were identified that contribute to enhanced shading capacity, and these traits were encapsulated into a single shading capacity metric. This was followed by the identification of the genetic loci underpinning variation in the core traits. The identified haplotypes can be used in breeding programmes to improve weed suppression by rice, thus contributing to sustainable agriculture.

Summary

- In modern rice farming, one of the major constraints is weed proliferation and the entailed ecological impact of herbicide application. This requires increased weed competitiveness in current rice varieties, achieved via enhanced shade casting to limit the growth of shade-sensitive weeds.
- To identify traits that increase rice shading capacity, we exhaustively phenotyped a rice diversity panel of 344 varieties at an early vegetative stage. A genome-wide association study (GWAS) revealed genetic loci underlying variation in canopy architecture traits linked with shading capacity.
- The screen shows considerable natural variation in shoot architecture for 13 examined traits, of which shading potential is mostly determined by projected shoot area, number of leaves, culm height and canopy solidity. The shading rank, a metric based on these core traits, identifies varieties with the highest shading potential. Five genetic loci were found to be associated with canopy architecture, shading potential and early vigour.
- Identification of traits contributing to shading capacity and underlying allelic variation will serve future genomic-assisted breeding programmes. Implementing the presented genetic resources for increased shading and weed competitiveness in

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rice breeding will make its farming less dependent on herbicides and contribute towards more environmentally sustainable agriculture.

KEYWORDS

allelic variation, genome-wide association study (GWAS), growth vigour, haplotype analysis, plant competition, rice diversity panel, shading capacity, shoot architecture

1 | INTRODUCTION

Rice feeds more than half of the world's population as a staple food (Kennedy & Burlingame, 2003; Wing et al., 2018). In traditional rice farming, seedlings are transplanted into flooded paddy fields. This works as a natural way to prevent weed infestation since it gives rice seedlings a size advantage in addition to flood-suppressed germination and the growth of weeds. This practice is increasingly problematic, both because of the high manual labour input (Chakraborty et al., 2017; Kumar & Ladha, 2011) and because global climate change is reducing the availability of fresh water not only for rice farmers but for the global agricultural sector (FAO, 2019; Oliver et al., 2019). The traditional rice farming system is transitioning towards direct-seeded rice, where rice seeds are directly sown into the fields. This practice drastically reduces the water requirement and labour input (Chauhan et al., 2017; Farooq et al., 2011; Kumar & Ladha, 2011). Besides all of its advantages, the major constraint for direct-seeded rice is the abundant proliferation of weeds (Rao et al., 2007; Xu et al., 2019). In direct-seeded rice (DSR) practice, rice seedlings are directly competing with weeds as they lose their seedling size advantage. Waterlogging cannot be applied to suppress emerging weeds, as most modern rice cultivars do not germinate under water (Chauhan, 2012; Ghosal et al., 2019; Kretschmar et al., 2015). Currently, weeds are suppressed with herbicides, leading to the evolution of herbicide-resistant weeds and groundwater pollution (Heap, 2014; Kraehmer et al., 2016). This creates a pressing need for the deployment of sustainable weed management options (Chauhan, 2012; Chauhan & Yadav, 2013; Mennan et al., 2012; Zhao et al., 2006a). One possible solution to this problem is to increase the weed competitiveness of the rice seedling (Chauhan, 2013; Dass et al., 2017; Dimaano et al., 2017; Johnson et al., 1998; Rao et al., 2007; Sakamoto et al., 2006; Zhao et al., 2007).

Just like their wild ancestors, shade-casting crop varieties compete with invading weeds by reducing the weed's access to full sunlight, thereby impeding their growth. However, the traits contributing to shading potential were neglected or even selected against in breeding efforts since tall plants and droopy leaves are generally considered undesirable because they make harvesting more difficult. Here we propose to develop weed-competitive rice varieties by selecting for an ideotype with faster growth and high shade-casting potential on proximate weeds. A large projected shoot area and therefore ground cover are associated with weed competitiveness (Caton et al., 2003; Dingkuhn et al., 2001; Haefele et al., 2004; Mennan et al., 2012; Namuco et al., 2009; Rao et al., 2007; Zhao et al., 2006b, 2007). In addition, a high number of leaves and tillering capacity, as well as

plant biomass and early vigour are advantageous for competition against weeds (Haefele et al., 2004; Mahajan & Chauhan, 2013; Namuco et al., 2009; Zhao et al., 2006a), but these are not specific architecture traits.

Shoot architecture traits that help plants gain an advantage over their neighbours through light competition include increased leaf area, increased planar angle of leaves and tillers and leaf droopiness (Andrew et al., 2015; Brainard et al., 2005; Dingkuhn et al., 1999; Mahajan & Chauhan, 2013; Seavers & Wright, 1999; Worthington & Reberg-Horton, 2013). Accelerated vertical growth might provide an additional advantage for outcompeting neighbours, yet plant height has been strongly selected against during the green revolution of most cereals, including rice. Indeed, increased shading potential of the crop has a clear potential for improvement towards sustainable weed suppression (Pantazopoulou et al., 2021; Peerzada et al., 2017; Seavers & Wright, 1999), which also applies for cereal canopies, as has been shown for wheat and other cereals where a rapidly closing crop canopy achieved through higher planting density and/or uniform planting pattern depleted weeds from access to light (Chauhan & Abugho, 2013; Chauhan et al., 2011; Marín & Weiner, 2014; Ottis & Talbert, 2007; Park et al., 2003; Weiner et al., 2010; Wolfe et al., 2008; Wu et al., 2021). The critical period of weed competition in rice is from the moment of sowing up to at least six weeks after sowing in a DSR system (Abdullah Al Mamun, 2014; Azmi et al., 2007; Chauhan & Johnson, 2011; Mennan et al., 2012; Raj & Syriac, 2017). Especially in the context of DSR, shading by the crop canopy would have to occur early in the season, and especially seedling vigour would substantially reduce weed growth (Mahajan & Chauhan, 2013; Subedi et al., 2019; Zhao et al., 2006a). A consequence of such early weed suppression, in addition to less need for herbicides, would be an increased yield at the harvest stage of the crop (de Vida et al., 2006; Laca et al., 2006; Mahender et al., 2015; Namuco et al., 2009; Subedi et al., 2019; Zhao et al., 2006a).

Building on the idea to increase shading for improved weed competitiveness, here (1) we phenotyped a rice diversity panel of 344 globally distributed varieties where we recorded 13 quantitative traits. Based on these, (2) we determined key architectural characteristics of shading potential in the early growth phase. (3) We combined these core traits into one parameter to develop the shading rank, where the rice varieties were ranked for their shading potential. (4) A genome-wide association study (GWAS) revealed association with eight genetic loci for traits contributing to shade potential. The results of this study form a primer for the identification of alleles contributing to increased shading and early plant vigour.

2 | MATERIALS AND METHODS

2.1 | Plant material

Three hundred forty-four Asian rice (*Oryza sativa*) varieties were used out of the rice diversity panel 1 (RDP1) (Eizenga et al., 2014) and one *Oryza glaberrima* variety (TOG7192) was included. The RDP1 is a collection of homozygous varieties from 82 countries. The panel includes landraces and elite rice cultivars from five subpopulations: *indica* and *aus* (of the Indica group), *tropical japonica*, *temperate japonica* and *aromatic* (comprising the Japonica group) and the *admixture* group (Liakat Ali et al., 2011; Zhao et al., 2011). Detailed information on the full panel is provided in Table S1.

2.2 | Growth conditions

Rice plants were grown in the greenhouse facilities of the International Rice Research Institute (IRRI) in the Philippines from October 2017 to April 2018. Temperatures ranged from 37°C during the day to 27°C at night, with a relative humidity of 75% and 80%, respectively, and a photoperiod of 11 to 12 h. The experiment followed a randomised block design, with the four replicate blocks separated in time. Each block contained three individual plants for each of all the investigated varieties, of which two were measured and harvested, and the third served as a backup in case of any potential failure. Plants were grown in single pots at a 30 cm × 30 cm distance. In the first experiment, seeds received from the IRRI gene bank were exposed to 40°C for 5 days to break dormancy, followed by 24 h at 21°C. For germination, seeds were put in Petri dishes on wet filter paper and incubated at 32°C for 24 h. Seeds were planted directly on the soil: four seeds were placed per pot (diameter of 16 cm and 13 cm high, without drainage holes), filled with sterilised clay-loam field soil mixed with NPK fertiliser (with 46/18/60 g per kg soil) and covered with a thin

layer of soil. From planting onwards, the soil was kept moist. Seven days after sowing (DAS), surplus seedlings were removed, retaining one seedling per pot. At 14 DAS, fertiliser with 50% N of the concentration of the first application was added. From 15 DAS onwards, the layer of water was maintained for water-logged conditions.

2.3 | Phenotyping

At 28 DAS, the following traits were measured manually: number of leaves and tillers, plant height, culm height and length of longest leaf. Plants were photographed from the top and side using two digital cameras in a fixed imaging set-up. The shoot was harvested, and dry weight was recorded after 48 h of drying at 70°C (IRRI, 2013). In Table 1, detailed evaluation methods for each trait are given. Dataset S1 contains the raw data for each replicate, and Table S2 provides the results of statistical analysis on phenotypic trait values. Figure S1 depicts scatter plots for all pair-wise phenotype comparisons.

2.4 | Data processing and statistical analysis

For the analysis of RGB images, an automatised image analysis pipeline was established using the open-source, Python-based PlantCV software (PlantCV version 3.7) (Fahlgren et al., 2015; Gehan et al., 2017). The script was optimised for monocots to extract values for shoot area, hull area and perimeter. The original Python script can be accessed at <https://plantcv.readthedocs.io/en/stable/>, and the customised Jupiter notebook used in this study is given in Dataset S2. Tiller angle, leaf angle and leaf erectness were measured using the free ImageJ software (<https://imagej.nih.gov/ij/>). Tiller angles were taken between the two outermost tillers and the culm, respectively. Similarly, leaf angles were recorded between the second and third youngest leaves and the culm. Leaf droopiness was measured on the

TABLE 1 Description of 13 investigated shoot architecture and growth traits in rice, with unit of measure and method of measurement.

Trait	Unit	Description
Number of leaves		Number of all visible green leaf blades
Number of tillers		Number of side branches classified as tillers as soon as it splits off the culm, having two leaves
Total plant height	cm	Height from soil to the straightened topmost leaf tip
Culm height	cm	Mother stem—from soil to highest node, where youngest leaf blade bends off
Leaf length	cm	Length of longest leaf blade
Projected shoot area	cm ²	All green leaf area projected from top view
Convex hull area	cm ²	Smallest area enclosing outermost leaf tips
Shoot perimeter	cm	Outline of the projected shoot area
Leaf initiation angle	°	Angle between culm and leaf blade initiation measured for second and third leaf
Tiller angle	°	Angle between the culm and tillers, measured for the left and right outermost tillers
Leaf droopiness	°	Interception angle of two tangents aligned to initiation and tip of leaf blade measured for second and third leaves
Dry weight shoot	g	Dry matter of shoot biomass after drying in oven at 70 C for 48 h
Solidity		Ratio of projected shoot area divided by convex hull area

same leaves, defined as the interception angle of two tangents aligned to the initiation and the tip of the leaf blade.

In the first block, 62 varieties were excluded as their position within the greenhouse received shading. In the later three blocks, we did not use this partially shaded area of the greenhouse anymore to ensure equal light conditions for all the studied plants. Prior to statistical analysis, the raw data were curated for outliers (using $1.5 \times \text{IQR}$ from the mean), and the mean was calculated out of the eight replicates (four blocks with two plants each). Statistical analysis such as ANOVA, Pearson correlation and hierarchical clustering were performed using R (R Version: 3.6.1-1bionic; R Core Team, 2020) and the online tool MVapp <https://mvapp.kaust.edu.sa> (Julkowska et al., 2019). The Pearson correlation coefficients between traits were calculated using raw data. Trait values were normalised per trait using the Fisher z-transformation and scaled prior to clustering by applying Ward D2 hierarchical clustering. For traits that had a correlation $>.5$, one core trait was chosen: shoot area, leaf number, solidity, culm height, leaf angle, tiller angle and leaf droopiness. The shading rank was calculated as follows:

First, trait values were normalised:

$$t_{\text{variety}}^n = \frac{t_{\text{variety}} - \min(t_{\text{variety}})}{\max(t_{\text{variety}}) - \min(t_{\text{variety}})} \times 100,$$

where t_{variety} is the value of a certain trait measured for a certain plant in the investigated population, and min and max are the minimum and maximum values of the measured trait in this population, with the normalised values ranging from 0 to 100.

Next, for each variety, a shading score was calculated:

$SS_{\text{variety}} = \sum_{\text{coretraits}} t_{\text{variety}}^n$ where \sum is the sum of the normalised values of the core traits. From this, we derive the shading rank (SR), which is the rank given to each variety according to its SS, ordering the varieties from 1 (lowest) to 344 (highest). The list of 344 varieties with their normalised core trait values, the sum of normalised core trait values, and their shading rank can be found in Table S3.

2.5 | GWAS

2.5.1 | Phenotype data

Mean values of all phenotypes were included, only *O. glaberrima* TOG7192 was excluded. We tested for normal distribution across the recorded traits prior to running the GWAS. Table S4 includes the list for all 344 varieties with 13 shoot trait values (as the mean value out of eight replicates) used for GWAS, that were cross-correlated in Figure S1.

2.5.2 | Genotype data

We used the imputed HDRA with 4.8 M SNPs from 3010 *O. sativa* varieties, including RDP1, RDP2 and NIAS (McCouch et al., 2016), and

the 3000 Rice Genomes data set (D. R. Wang, Agosto-Pérez, et al., 2018), available at <http://ricediversity.org/data/index.cfm>. The data was curated by filtering for unique SNPs with a 90% minimum count and a minor allele frequency $\geq 5\%$. For the 344 phenotyped varieties, this resulted in a total of 1.7 M SNPs used for the GWAS. As an average genome-wide linkage disequilibrium (LD) decay in rice, we used previously calculated values (Huang et al., 2010; Zhao et al., 2011).

We used two different software packages for GWAS just to confirm the robustness of any identified associations, and only loci that were identified by both methods were followed up on afterwards. The first is an R package (R version 3.6.1) of Genomic Association and Prediction Integrated Tool (GAPIT) (Li et al., 2014; Tang et al., 2016; Wang, Tian, et al., 2018). We employed a mixed linear model (MLM) (Yu et al., 2006) with the optimal number of principal components based on the calculated Bayesian information criterion (BIC) for each trait, including as coefficients a kinship matrix (K-matrix) based on clustering analysis to account for genetic relationships between individuals, together with the population structure (Q-matrix). The second software package is lme4QTL. GWAS was performed as described in Ziyatdinov et al. (2018), taking population structure into account by using a kinship matrix, and calculated with the `cov()` function in R 3.6 (Figure S2). The decomposition matrix to correct for population structure was made by following the lme4QTL protocol and using the `relmatLmer()`, `varcov()` and `decompose_varcov()` functions in order. The obtained decomposition matrix, together with the traits and binary SNP matrix, is then used in the `matlm()` function to calculate the significance and effect per SNP. The full list of detected significant SNP associations can be found in Dataset S3. As a confirmation for the reliability of SNP trait associations, we compared the results of the two methods applied here. The Manhattan plots for all investigated traits using lme4QTL can be found in Dataset S4 and for GAPIT in Dataset S5). An exact overlap was not expected, as there are minor differences in the calculation of the SNP-trait associations: GAPIT uses MLM to create the SNP-trait association and a principal component analysis that we set to “optimal” to calculate the optimal number of PCs needed to describe the variation in population structure. The lme4QTL is a mixed model solver (Linear Mixed Effects 4 QTL) that can use a kinship matrix as a covariance to control for population structure. The narrow-sense heritability (h^2) of the analysed traits was calculated with GAPIT (Table S5). To set the significance threshold, the rather conservative Bonferroni correction was applied, calculated by the $-\log_{10}(p\text{-value of } .05/\Sigma \text{ SNPs})$, which corresponds to $-\log_{10}(0.05/1.700.000) = -7.53$ for the imputed HDRA data set. To examine the GWAS model performance and estimate possible model overfitting, QQ plots were generated (Dataset S6).

2.6 | Post-GWAS analysis

For all follow-up analysis, the output of the GWAS using the lme4QTL method on the raw, untransformed phenotype data was used.

2.6.1 | Locus definition

Loci that were detected with both methods (lme4QTL and GAPIT) were considered most robust and subsequently used as a basis for the selection of loci of interest and follow-up analysis. A locus of interest was determined if several significantly associated SNPs were found in close proximity. Single SNPs passing the threshold were neglected since whole-genome sequencing data provides sufficient markers in each LD block. Rice has a low rate of LD decay, which makes the identification of causal genes more difficult (Wang et al., 2020). Therefore, the local LD analysis was used to define LD clumps surrounding the index SNPs using LD clumping in PLINK (<http://zzz.bwh.harvard.edu/plink/>). We set three selection criteria: strong LD between SNPs, a p -value threshold of .01 and a physical distance of 250 kb, given with the R^2 value. We considered SNPs with $-\log^{10}(p\text{-value}) > 5$ as index SNPs to perform the analysis and clumped SNPs with a p -value > 4 . For the determination of loci of interest, we focused on the core traits culm height, shoot area, solidity and number of leaves. For culm height and number of leaves, single significant SNPs were not found to be surrounded by other significant SNPs within LD and therefore did not meet our selection criteria. Since dry weight was highly correlated with the traits of branchiness, the loci found for dry weight were included as representatives for branchiness, and similarly, the loci for plant height were included as representatives of height-related traits.

2.6.2 | Gene models

Genetic regions covered by significant SNPs were searched for candidate genes using two different gene annotation models, which were then merged: the Michigan State University (MSU; October 31, 2011—Release 7; <http://rice.plantbiology.msu.edu/>) and the Rice Annotation Project Database (RAP-DB; March 24, 2020; <https://rapdb.dna.affrc.go.jp/>). Other data resources used were the gene ID converter (<https://rapdb.dna.affrc.go.jp/tools/converter>), GALAXY—rice genome browser (http://13.250.174.27:8080/?tool_id=getgenes&version=1.0.0&__identifier=pxuu9t4bnk) and SNP seek (<http://snp-seek.irri.org/>).

2.7 | Haplotype analysis

To facilitate the identification of candidate genes within the detected loci related to canopy architecture, we performed haplotype analysis spanning the coding sequence regions of the genes within each locus. Since haplotype analysis can only be performed for annotated genes, SNPs that are not in a coding sequence cannot be included in haplotype analysis. For each locus, we used the combined gene model annotation (MSU and RAP-DB) to identify the coding sequences of the individual genes (Table S6). Subsequently, all SNPs within the coding sequence region were compiled into one haplotype, and all varieties were grouped based on their haplotype sequence, with haplotypes represented by two or less varieties being excluded from

the analysis. If significant SNPs were too far away to be within LD, they were not defined as loci and were not included in further haplotype analysis. Based on the haplotype grouping for each coding sequence, we performed an ANOVA followed by a post-hoc test for significant differences between the haplotypes for each measured trait. The individual haplotypes are represented by A/T, where A stands for a variant of the reference accession and T for any alternative variant. Dataset S7 shows haplotype groups for all determined loci of interest with their phenotype effects for 13 investigated shoot traits. For more information on all investigated loci, consult Dataset S8, which contains the full list of haplotype sequences within the defined loci of interest.

2.8 | Canopy shading experiment

Rice plants were grown in the greenhouse facilities of Utrecht University, The Netherlands, in February 2021. Temperatures were set to 29°C during the day and 25°C during the night, with a photoperiod from 8 a.m. to 8 p.m., with a minimal light intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and artificial light (Valoya, Model Rx400 500 mA 5730, Spectrum AP673L) switching on if sunlight dropped below 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Automatic watering kept the soil water-saturated. The selected *O. sativa* varieties were Shim Balte, Mudgo, Della and Luk Takhar, with shading ranks of 344, 330, 49 and 1, respectively. The germination protocol was followed as described above. Four plants were grown per pot in each of the corners of a square pot (10 × 10 × 11 cm) in a substrate mix of black soil, vermiculite and sand in a ratio of 5:3:2 together with 6 g of Osmocote slow-release fertiliser and 1 L of Yoshida nutrient solution per kg of substrate. Pots were arranged at a distance of 10 cm in mixed plots. The experiment units (the eight plants that were measured) were surrounded by bordering plants to avoid border effects on the experimental units. Light intensity (photosynthetic active radiation [PAR] of the 400–700 nm waveband) was measured at ground level between rice plants (with six measurements in each of the three replicates) and above the plants for reference at the same time to calculate light extinction. PAR values can be found in Table S7.

3 | RESULTS

3.1 | Shoot architectural variation between rice varieties

In order to evaluate the variation in shading potential within the rice diversity panel (Table S1), we measured 13 traits on 4-week-old seedlings in the screenhouse (Figure 1, Table 1, Table S2).

Substantial variation was observed for all measured traits among the varieties belonging to different subpopulations (Figure 1; Table S2). The *indica* subpopulation showed the highest dry weight, number of leaves and number of tillers, followed by the *aus* subpopulation, and *aromatic*, *tropical* and *temperate japonica* ranked lowest for

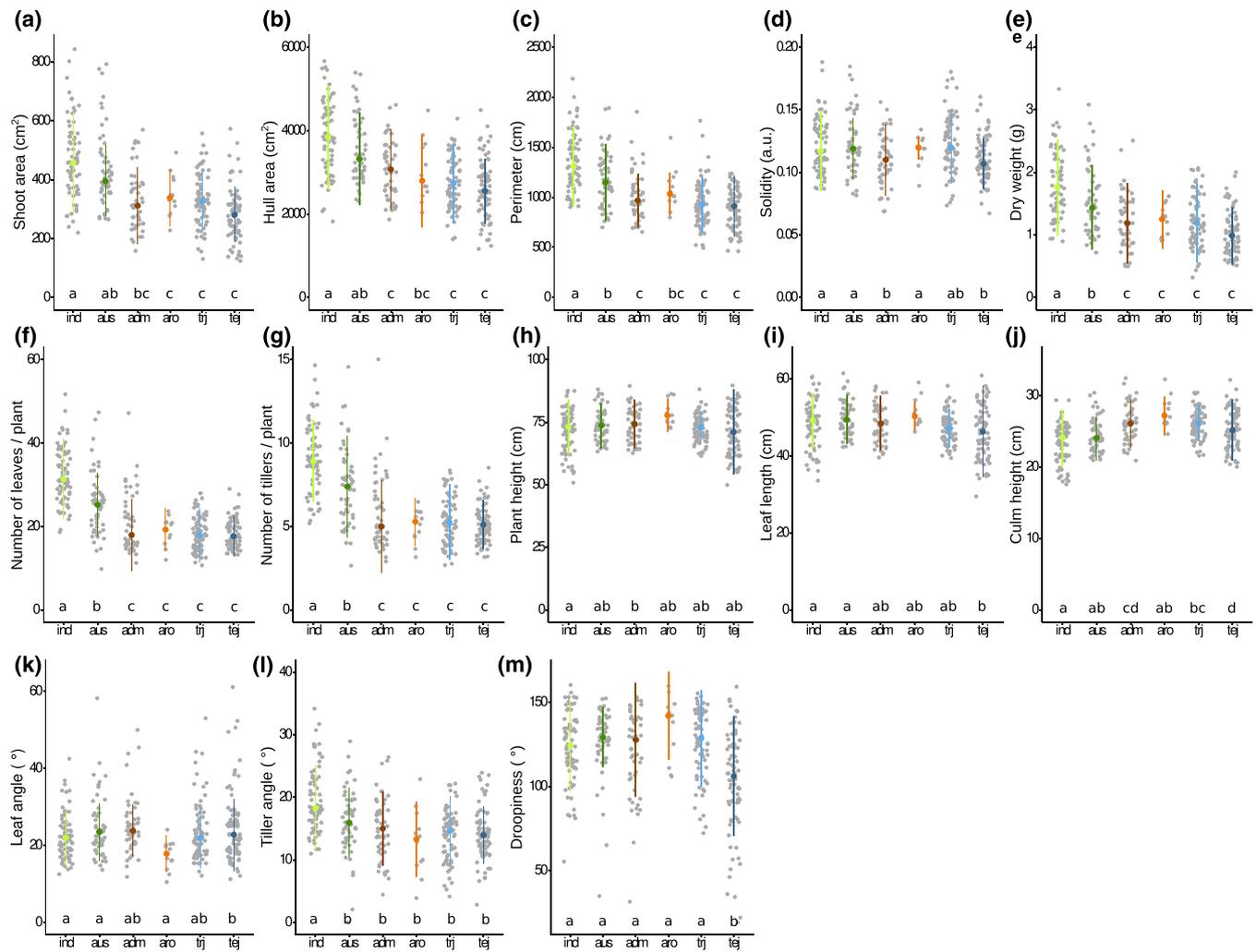


FIGURE 1 Shoot traits in rice differ between subpopulations. Distribution of investigated shoot traits in the screened diversity panel. The plots represent the trait value (y-axis) observed for varieties grouped according to different subpopulations on x-axis. (a) Shoot area [cm²], (b) hull area [cm²], (c) perimeter [cm], (d) solidity, (e) dry weight [g], (f) number of leaves/plant, (g) number of tillers/plant, (h) plant height [cm], (i) leaf length [cm], (j) culm height [cm], (k) leaf angle [°], (l) tiller angle [°] and (m) droopiness [°]. Each data point represents the mean out of eight replicates for each of the 344 varieties. The colours represent different groups of subpopulations: *ind*, *indica*; *aus*, *adm*, *admixed*; *aro*, *aromatic*; *trj*, *tropical japonica* and *tej*, *temperate japonica*. The letters in the graphs represent the significantly different groups, as determined with Tukey's HSD with *p*-value < .05, error bars indicate standard deviation, horizontal bars indicate the median. Mean values for all 13 traits and the sum of the normalised traits including results for Tukey's pairwise post hoc test can be found in Table S2.

these parameters (Table S2). Shoot and hull area were also observed to be higher in *indica* and *aus* subpopulations, intermediate in *aromatic* subpopulation, and lowest in *japonicas* and *admixture* subpopulations. *Indica* and *aus* on average develop the most compact shoots (highest solidity), contrasting with the low solidity of *japonicas* and *admixed*. In culm height, *indica* and *aus* were shortest, and *temperate japonica* and *admixed* subpopulation were tallest. When taking the entire diversity panel of 344 varieties, five traits (shoot area, hull area, solidity, plant height and dry weight) already showed a significant difference between the individual varieties at 4 weeks after sowing (Table S2). When grouped together in subpopulations, all traits showed significant differences between subpopulations (Table S2). Overall, it appears that relatively large variation between subpopulations was

observed for traits related to area and branchiness, whereas traits related to height showed only little variation between subpopulations. These differences are clearly determined by differences in genetic background since the growth conditions were constant. The high variation observed for traits related to shading potential suggests that the investigated rice diversity panel offers the genetic variation needed to inspire improvement of shading potential in elite-breeding varieties.

3.2 | Correlation of shoot architectural traits

To explore the relationship between individually measured traits and determine which traits are independent of each other, we performed a

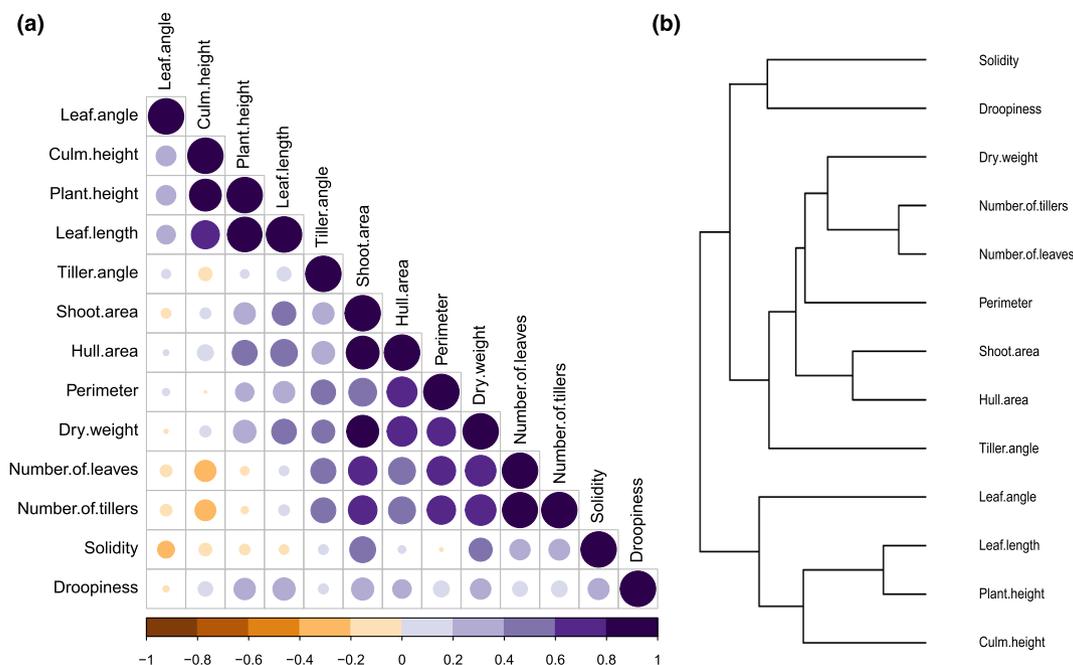


FIGURE 2 Correlation and clustering of 13 shoot growth and architecture traits in rice highlights trait groups that define the core trait groups. (a) Pearson correlation coefficients between traits. The colour and size of the circles reflect the strength of the correlation (see colour scale below diagram). (b) Hierarchical cluster analysis. Traits are clustered using ward, D2 method. Rows represent 13 studied shoot traits, the length of branches is proportional to the distance in clustering. The values of individual samples are normalised per trait using Z-Fisher transformation scaled prior to clustering. Based on a cut off at seven clusters and together with the correlation coefficients, we grouped together the traits into defined core groups.

Pearson correlation analysis (Figure 2a, Figure S1). Shoot area and hull area showed a strong positive correlation with shoot dry weight. Leaf and tiller numbers were highly correlated with shoot area and dry weight. Height-associated traits, such as plant height, culm height and leaf length, were positively correlated with each other. On the other hand, a negative correlation was found between culm height and the number of leaves and tillers. Solidity, leaf angle, tiller angle and droopiness did not display strong correlations with other measured traits.

To examine the types of canopy architecture exhibited within the rice diversity panel, we performed hierarchical clustering (Figure 2b), resulting in seven trait clusters. The clustering shows how traits are grouped together according to the patterns observed across all rice varieties. Taking the correlation and clustering analyses together, we can classify core groups of traits: area-related (shoot area, hull area, perimeter), branchiness (number of leaves and tillers and dry weight), height-related (plant and culm height and leaf length), solidity, leaf angle, tiller angle and droopiness (Table 2).

3.3 | Defining ‘shading potential’ from shoot architecture traits

The shading potential of a plant expresses the effectiveness with which it can cover ground area. The traits chosen to be included in a rank that stands for shading potential were included based on literature research (Andrew et al., 2015; Caton et al., 2003; Dingkuhn

TABLE 2 Core groups of shoot traits in rice. Measured shoot architecture traits were grouped based on their correlation and clustering. For core groups with multiple traits, we have selected a representative trait as the core trait, indicated in bold.

Core groups	Measured shoot architectural traits
Area	Projected shoot area, convex hull area, perimeter
Branchiness	Number of leaves, number of tillers, dry weight
Height	Culm height, leaf length, plant height
Solidity	Solidity
Leaf angle	Leaf angle
Tiller angle	Tiller angle
Droopiness	Droopiness

et al., 2001; Haefele et al., 2004; Mahajan & Chauhan, 2013; Mennan et al., 2012; Namuco et al., 2009; Rao et al., 2007; Worthington & Reberg-Horton, 2013; Zhao et al., 2006b, 2007) together with a general understanding of plant architecture and light extinction. From the large set of shoot architectural traits, we defined core traits based on clustering and correlation analysis (Figure 2a), revealing which traits are closely linked and would therefore be overrepresented if all taken into account with the same weight for ranking the varieties. Core traits are chosen as representative of a correlating group of traits. To quantify shading potential, we ranked varieties for the sum of the core traits contributing to shading potential (projected shoot area, number of leaves, solidity, culm height, leaf angle, tiller angle and leaf

TABLE 3 Shading rank for 10 highest and 10 lowest ranking rice varieties, and for varieties of special interest (Mudgo, IR 64-21, Nipponbare and Della) with normalised core trait values (between 0 as lowest and 100 highest) and the sum of the core traits. Varieties in bold are visualised in Figure 3. The shading rank ranges from 344 as the highest and 1 as the lowest shading. The list of shading ranks for the entire panel can be found in Table S3. Subpopulations: *ind*, *indica*; *aus*, *adm*, *admixed*; *aro*, *aromatic*; *trj*, *tropical japonica* and *tej*, *temperate japonica*; *norm*, normalised trait value without unit; *SUM_norm_traits*, sum of normalised trait values.

Variety	Subpopulation	Shoot area norm	Number of leaves norm	Solidity norm	Culm height norm	Leaf angle norm	Tiller angle norm	Droopiness norm	SUM_norm_traits	Shading rank
SHIM BALTE	<i>aus</i>	78	85	73	86	94	65	79	561	344
SZE GUEN ZIM	<i>ind</i>	100	100	95	38	15	55	67	470	343
PARAIBA CHINES NOVA	<i>ind</i>	77	55	51	64	25	100	90	462	342
P 737	<i>aus</i>	91	56	69	84	42	49	68	458	341
SHIRKATI	<i>aus</i>	93	61	68	51	8	85	80	446	340
SABHARAJ	<i>ind</i>	94	78	63	54	23	57	73	443	339
PAUNG MALAUNG	<i>aus</i>	89	56	97	52	16	45	85	440	338
NIRA	<i>ind</i>	80	64	56	47	32	70	82	431	337
SATHI	<i>aus</i>	67	59	66	73	22	52	81	420	336
MTU9	<i>ind</i>	86	46	57	79	19	48	82	417	335
MUDGO	<i>ind</i>	73	30	57	79	20	53	95	407	330
IR 64-21	<i>ind</i>	16	59	41	13	16	32	78	254	74
NIPPONBARE	<i>tej</i>	19	25	52	25	13	42	77	253	73
DELLA	<i>trj</i>	11	6	12	38	66	46	56	234	49
COCODRIE	<i>trj</i>	10	11	22	39	23	26	38	168	10
L 202	<i>trj</i>	1	10	9	27	14	44	61	166	9
TRIOMPHE DU MAROC	<i>tej</i>	2	10	51	52	22	25	2	165	8
S 4542 A 3-49B-2-12	<i>trj</i>	4	8	7	48	5	43	43	159	7
TAINAN IKU 487	<i>tej</i>	5	24	38	36	12	19	19	154	6
PI 298967-1	<i>adm</i>	5	11	1	42	17	34	34	143	5
SHIROGANE	<i>tej</i>	4	17	14	19	12	34	43	142	4
BUL ZO	<i>tej</i>	10	8	20	45	22	21	11	137	3
GUINEANDAO	<i>adm</i>	10	14	9	38	8	40	9	127	2
LUK TAKHAR	<i>tej</i>	3	8	26	17	5	44	0	103	1

droopiness, bold in Table 2). Each of the traits was then normalised so as to become comparable across traits that have different magnitudes and measured units by rescaling the values to a range from 0 to 100 while keeping the relative differences of trait values between different varieties unchanged, and these relative differences are also reflected in the sum of the normalised trait values. Varieties were then ranked according to their sum of normalised trait values, from 344 (highest) to 1 (lowest), resulting in the shading rank (for detailed information, see Section 2.4). The resulting shading ranks within this diversity panel are shown in Table S3. Since the diversity panel was evaluated 28 days after sowing, a large shoot size in high-ranking varieties also indicates rapid growth and seedling vigour. From the 25 highest-ranking varieties, 14 belong to the *indica* subpopulation and eight to *aus*. Low-ranking varieties in terms of shading potential include widely grown varieties such as IR 64 and Nipponbare

(Acevedo-Siaca et al., 2020), ranking 74th and 73rd, respectively (Table 3). This suggests that some of the current elite rice varieties could have a rather poor shading potential, and through breeding with varieties from *indica* and *aus* subpopulations, the shading potential and weed competitiveness can possibly be increased.

The distribution of the different varieties with respect to the core trait groups area, branchiness, height and solidity is shown in Figure 3, together with top images of representative varieties. None of the top-ranking varieties showed the highest values for all core shading traits (Figure 3), hinting at trade-offs between shading traits. For example, Sze Guen Zim ranks highest for shoot area and number of leaves but is one of the lower-ranking varieties for culm height. The variety with the highest shading rank (344), Shim Balte, has a very high number of leaves and solidity but has a close to average culm height. Mudgo reaches a rank of 340 despite its relatively low number of leaves and

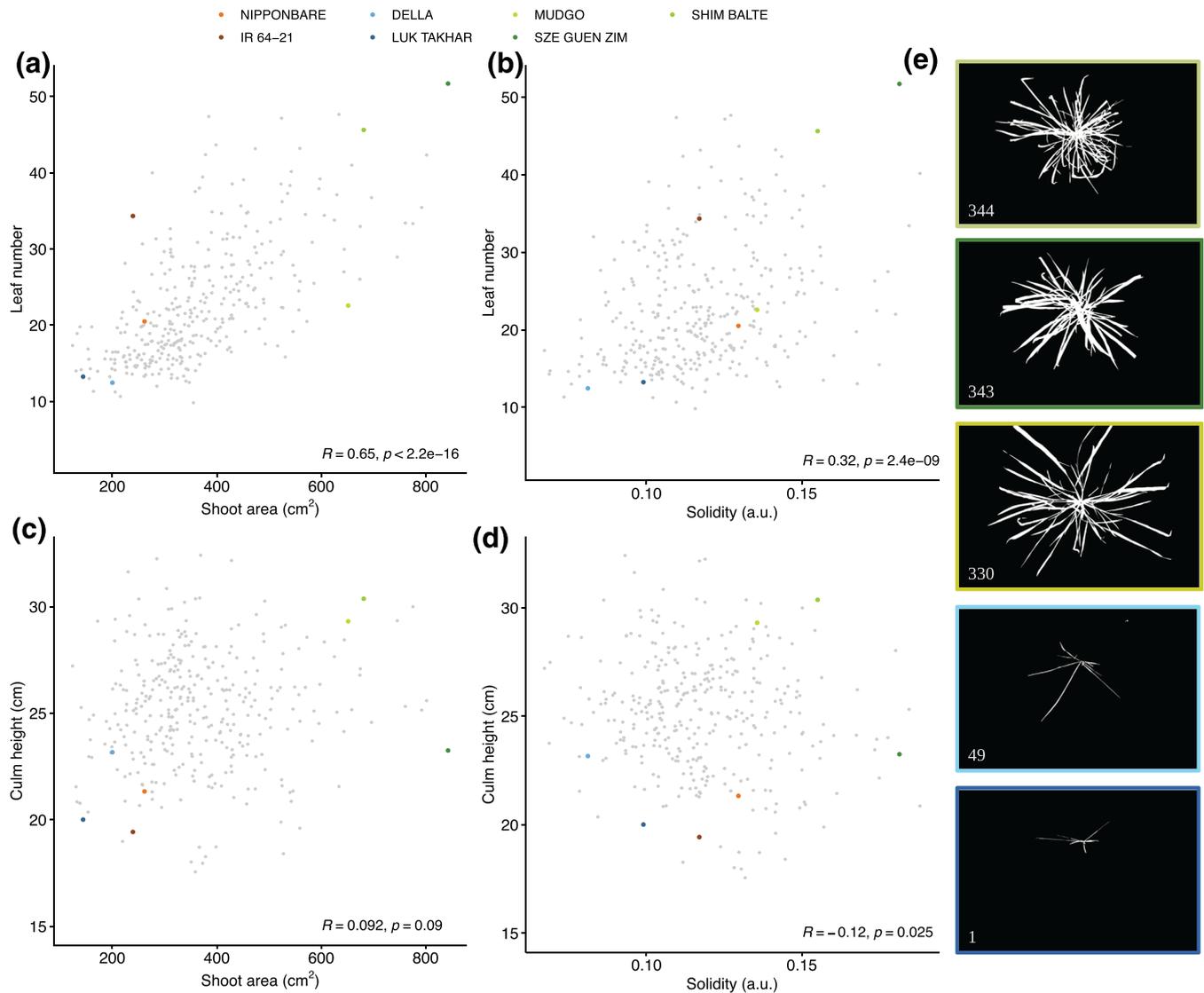


FIGURE 3 Shoot architecture traits associated with shading capacity and projected shading in the investigated rice diversity panel. (a–d) Scatter plots showing the distribution of 344 rice varieties in pair-wise combination of four core traits of the shading rank, shoot area, number of leaves, solidity and culm height. Representative high (344, 343 and 330) and low (49 and 1) ranking varieties together with Nipponbare (73) and IR 64-21 (74) are highlighted in colours. (e) Top view images of representative varieties, with colour coded frames. Numbers are respective shading ranks as found in Table 3.

solidity. At the other end of the spectrum, Della ranks 49 and is low for all traits except for culm height, whereas Luk Takhar (rank 1) shows low values for all core traits.

3.4 | Predicted competitive varieties are casting more shade

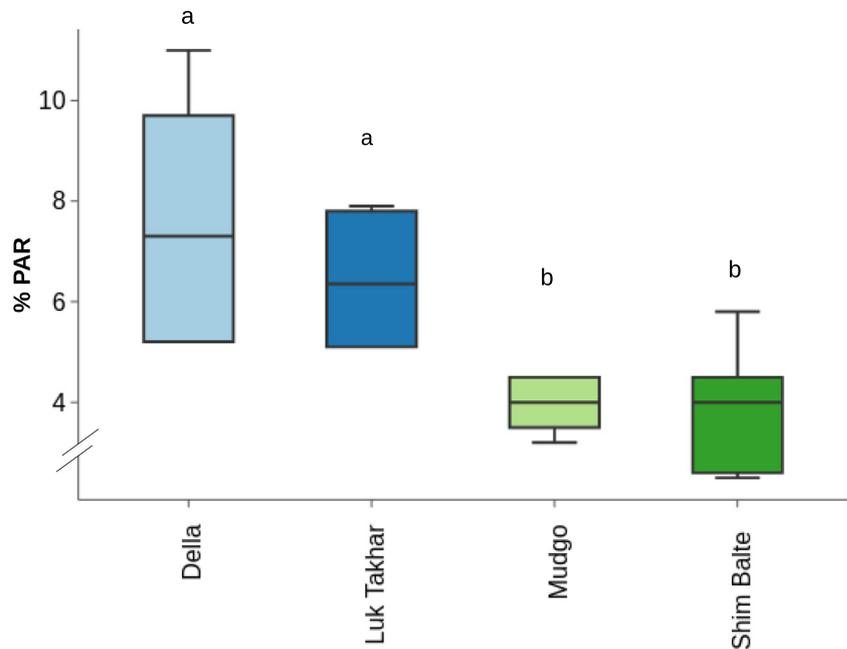
To validate our shading rank and assess functional shading capacity, we grew varieties with varying shading rank and evaluated them for canopy shading. We selected two of the predicted competitive (Shim Balte with a Shading Rank of 344 and Mudgo ranking 330) and two predicted non-competitive rice varieties (Luk Takhar ranking 1 and Della ranking 49) (Figure 4, Table 3). By measuring the light quantity

under the canopies of selected varieties (Table S4), we indeed observed strong shading by varieties with a high shading rank (Shim Balte and Mudgo) and less severe shading by varieties with a low shading rank (Luk Takhar and Della). This result validates our shading rank, at least for the varieties tested and the selection of shoot architecture traits to effectively predict shade casting.

3.5 | SNPs associated with seedling establishment and shoot architectural traits

The high phenotypic variability found in the studied diversity panel (Table S4), together with the high genetic variation (Wang, Longkumer, et al., 2018), provides a strong basis for a GWAS. We

FIGURE 4 Canopy shading capacity is consistent with shading rank of high and low ranking rice varieties. Significant difference in light extinction between canopies of different rice varieties at 5 weeks after sowing. The plot shows the reduction in light intensity (%PAR) measured at the ground level under the rice canopy compared to above the canopy, for different rice varieties on x-axis, where Della and Luk Takhar were classified as non-competitive (blue) with shading ranks of 49 and 1, respectively, and Mudgo and Shim Balte as competitive (green) with shading ranks of 330 and 344, respectively. Letters indicate significance (ANOVA with Tukey's pairwise comparison post hoc test $p < .05$). Measured PAR values (photosynthetic active radiation of 400–700 nm waveband) can be found in Table S7.



observed high narrow-sense heritability for all measured traits (Table S5). We investigated the genomic trait associations on the latest available SNP set at the time of analysis, with 4.8 M SNPs from 3010 *O. sativa* varieties (McCouch et al., 2016; D. R. Wang, Agosto-Pérez, et al., 2018), with two different software packages (lme4QTL (Ziyatdinov et al., 2018) and Genomic Association and Prediction Integrated Tool (GAPIT) (Tang et al., 2016; Wang, Tian, et al., 2018); see methods for a detailed description). The total list of p-values for SNPs association across all measured traits can be found in Dataset S3, resulting from the lme4QTL package.

Despite solidity being a very complex and likely a polygenic trait, the analysis revealed a strong association with 14 SNPs in the locus on chromosome 3 (Figure 5). Three genomic regions were associated with plant height, located on chromosomes 3, 5 and 6 (Figure 5). The peak on chromosome 3 was also detected for other height-related traits: culm height and leaf length (Dataset S4). Overall, the associations with culm height showed lower LOD scores (Dataset S4), and thus we followed up the loci in plant height. The results for droopiness reveal strong associations with SNPs on chromosomes 1 and 10, sharing the association on chromosome 1 with tiller angle (Figure 5, Dataset S4). Leaf angle could be associated with a highly significant peak of SNPs on chromosome 12. The associations between leaf or tiller number found for SNPs on chromosomes 11 and 12 were shared between these two traits (Dataset S4). These two loci were also found for dry weight. This suggests that the genetic components underlying the formation of new leaves and tillers might have a common genetic constituent, consistent with the high correlation in their phenotypes (Figure 2). The analysis for dry weight revealed significant associations on chromosomes 3, 7 and 12, overlapping with the associations found for shoot area (Figure 5). The strong accumulation of significantly associated SNPs on chromosome 1 were also found to be associated with solidity, shoot area and dry weight,

representing three of the core traits. When taking shading potential together as the sum of all core traits, a GWAS on this composite trait yielded a rather random pattern of SNP associations (Figure S4). This further highlights our earlier findings (Table 3), that shading can be achieved through various strategies, and shading potential, as such, is genetically a highly complex trait. Therefore, genetic mapping of shoot architecture components that contribute to shading capacity is an effective approach to identifying genetic components that contribute to shading and potential weed competitiveness.

3.6 | Identification of alleles associated with increased shading potential

The genomic regions that consisted of multiple SNPs above the Bonferroni threshold within the calculated local average LD (Table 4) were investigated in more detail. Because the traits related to canopy shading potential are the primary focus of this work, we prioritised the loci associated with culm height, shoot area, solidity and dry weight (Figure 5). In total, we determined six loci to be followed up with a haplotype analysis to identify specific alleles that could contribute to traits determining shading potential. By grouping varieties according to SNPs within one coding region and examining the phenotypic differences between identified haplotypes, we identified allelic variation associated with high shading potential (Figure 6). On chromosome 1, we found two loci, the first one for droopiness (Figure 6a) in the coding sequence for a GTP-binding protein (Os01g0225200), where one haplotype (hap03) had a significantly lower droopiness compared with all others. The second locus on chromosome 1 was found to overlap between shoot area, solidity and dry weight (Figure 6d–f) in a sequence encoding a protein with protein phosphorylation function (Os01g0810800). The haplotypes

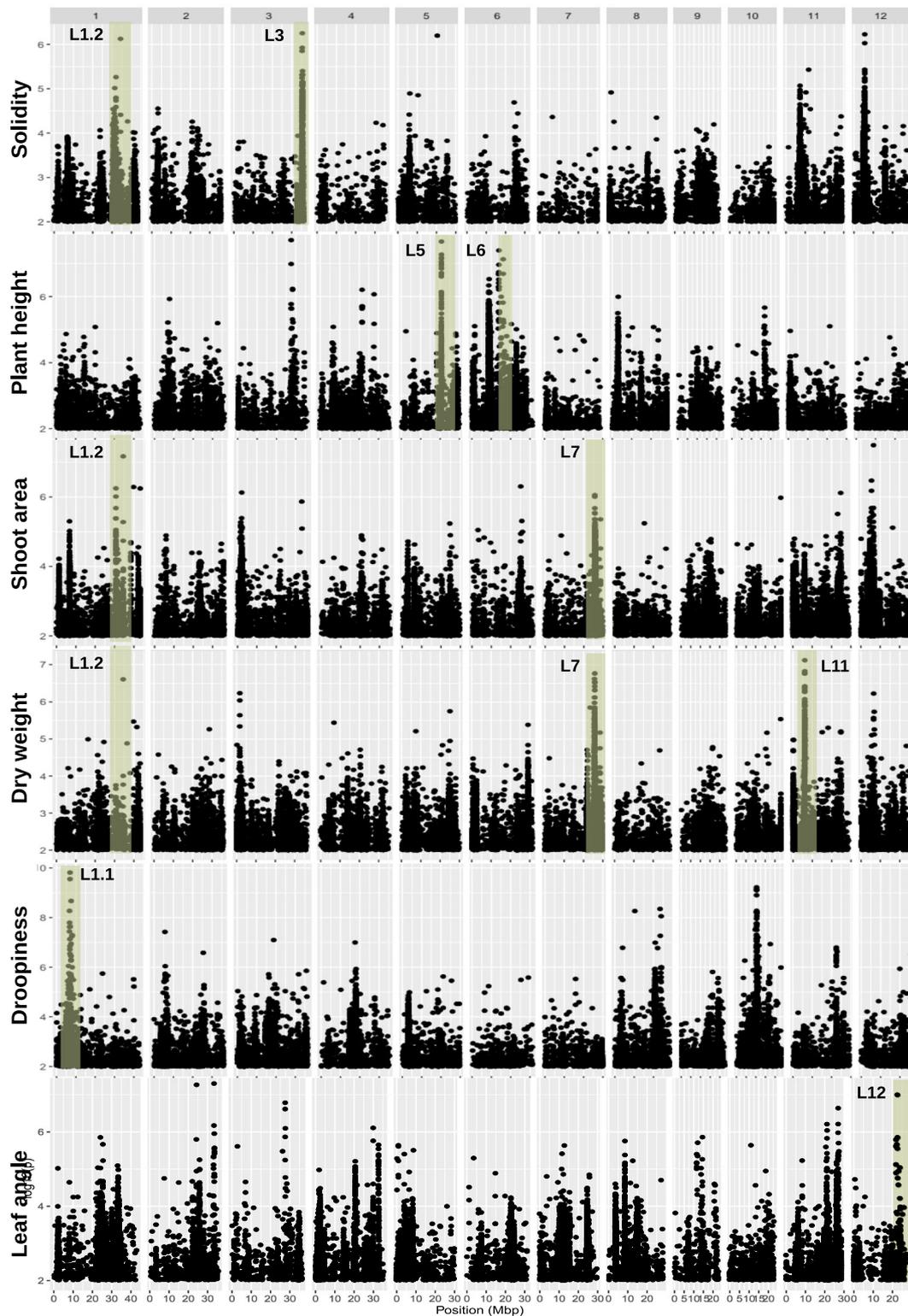


FIGURE 5 Genome-wide association study (GWAS) identifies the genetic regions underlying shoot architectural traits and seedling vigour in 4-week-old rice seedlings, reflecting the early vegetative growth stage. We used single-trait GWAS with a mixed linear model (MLM) for solidity, plant height, shoot area, dry weight, droopiness and leaf angle. The Manhattan plots depict the single nucleotide polymorphisms (SNPs) with minor allele frequencies (MAFs) $>.05$. Negative logarithmic p -values on the y-axis, for 1.7 M SNPs across the 12 rice chromosomes along the x-axis. Genomic regions highlighted in green are loci of interest (numbered L1.1-L12). These results were generated by using the software package lme4QTL.

TABLE 4 Summary of determined loci and genes of interest of core rice trait groups for shading potential with index SNPs (significant SNPs with LOD >5) and locus span in kb (clumped SNPs with LOD >4 in local LD up- and downstream), with the locus ID and gene annotation. Genes represented in Figure 6 are highlighted in bold. Full list of SNP positions in loci of interest with gene annotation and gene ontology categories can be found in Table S6. These results were generated by using the software package lme4QTL. Chr, chromosome; SNP, single nucleotide polymorphism; index SNP_ID, ID of identified index SNP within the span of the locus; Locus_ID, annotated for *Oryza sativa*.

Trait	Locus	Chr	Index SNP_ID	Locus span [kb]	Locus_ID	Gene annotation
Droopiness	L-1.1	1	1.01395336	49	Os01g0225200	Predicted protein; BP: GTP binding
Shoot area	L-1.2	1	1.07664139	6	Os01g0810800	Hypothetical conserved gene; BP: protein phosphorylation
Dry weight						
Solidity						
Solidity	L-3	3	3.35500735	404	Os03g0841800	GSK3/SHAGGY-like kinase
					Os03g0841850	Hypothetical protein.
					Os03g0843700	FAR1 domain containing protein
					Os03g0845000	Similar to Pirin-like protein
					Os03g0845700	Similar to RPB17 (fragment)
					Os03g0845800	Conserved hypothetical protein
					Os03g0848700	Coiled-coil, nucleotide-binding, and leucine-rich repeat protein
Plant height	L-5	5	5.20612311	59	Os05g0420500	Conserved hypothetical protein
					Os05g0420600	Cytochrome c
					Os05g0420900	Conserved hypothetical protein
Plant height	L6	6	6.13994152	240	Os06g0269300	TolB-like domain containing protein
					Os06g0346300	Acyl-CoA oxidase/oxidoreductase
Shoot area	L-7	7	7.25787749	146	Os07g0623200	Heavy metal transporter protein; ATPase, P-type
Dry weight		7			Os07g0623501	Hypothetical gene
		7			Os07g0623600	Similar to mRNA, clone: RTFL01-43-H20
Dry weight	L-11	11	11.6059294	23	Os11g0216000	Pyruvate kinase family protein
Leaf angle	L-12	12	12.085063092	1	Os12g0557800	Pentatricopeptide repeat domain containing protein

of three coding regions in locus 3 (Figure 6g-i), associated with solidity, were observed to have significantly lower solidity than the most abundant haplotype. These are annotated as a FAR1 domain-containing protein (Os03g0843700), Pirin-like protein (Os03g0845000) and a RPB17 fragment (Os03g0845700). Locus 5 (Figure 6j-k), associated with plant height, includes Cytochrome C (Os05g0420600) and a conserved hypothetical protein (Os05g0420900), where for both, the most abundant haplotype was linked to the shortest plants. In locus 7, associated with shoot area and dry weight, we found that only one gene (Os07g0623200, annotated as ATPase and heavy metal transporter protein) showed clear separation across the haplotypes, where all the non-reference haplotypes showed higher shading potential, indicated by higher shoot area and dry weight (Figure 6l,m). Within locus 11, associated with solidity and dry weight (Figure 6b,c), there is only one gene located, encoding a pyruvate kinase family protein (Os11g0216000). We found that the second most abundant haplotype was associated with increased shading due to the higher dry weight of varieties that shared this specific combination of SNPs.

We then summarised these main haplotypes into a table where we express their contribution (positive/neutral/negative) to the

expression of the core traits that we used to compile the shading rank. This facilitates a direct impression of how specific alleles, detected as haplotypes as mentioned above, affect all the core traits that together determine the shading rank (Figure 7). As such, this would facilitate decisions on which alleles would be expected to be a potential source for improving shading potential in elite varieties. From this integrative table, it becomes clear that a number of haplotypes are highly desirable. Hap2 from locus L-1.1 (Os01g0225200), hap2 from locus L-1.2 (Os01g0810800), hap3, hap4 and hap5 from locus L-3 (Os03g0843700) and hap3 from another gene in locus L-3 (Os03g0845000) are all alleles that positively affect values of multiple core traits that contribute to shading potential without negatively affecting any of the other core traits. Since these alleles are not highly abundant in the studied population (Figure 6), it is highly likely that they are not represented in the current elite varieties. From this table, it also becomes clear that allelic effects on trait expression are quite consistent between shoot area, dry weight and leaf number. Alleles that are positive for these traits, however, are often negative or neutral for culm height. Furthermore, all alleles that seem to stimulate culm height reduce expression of (multiple) other core traits, indicating that within this population there is little potential for improvement

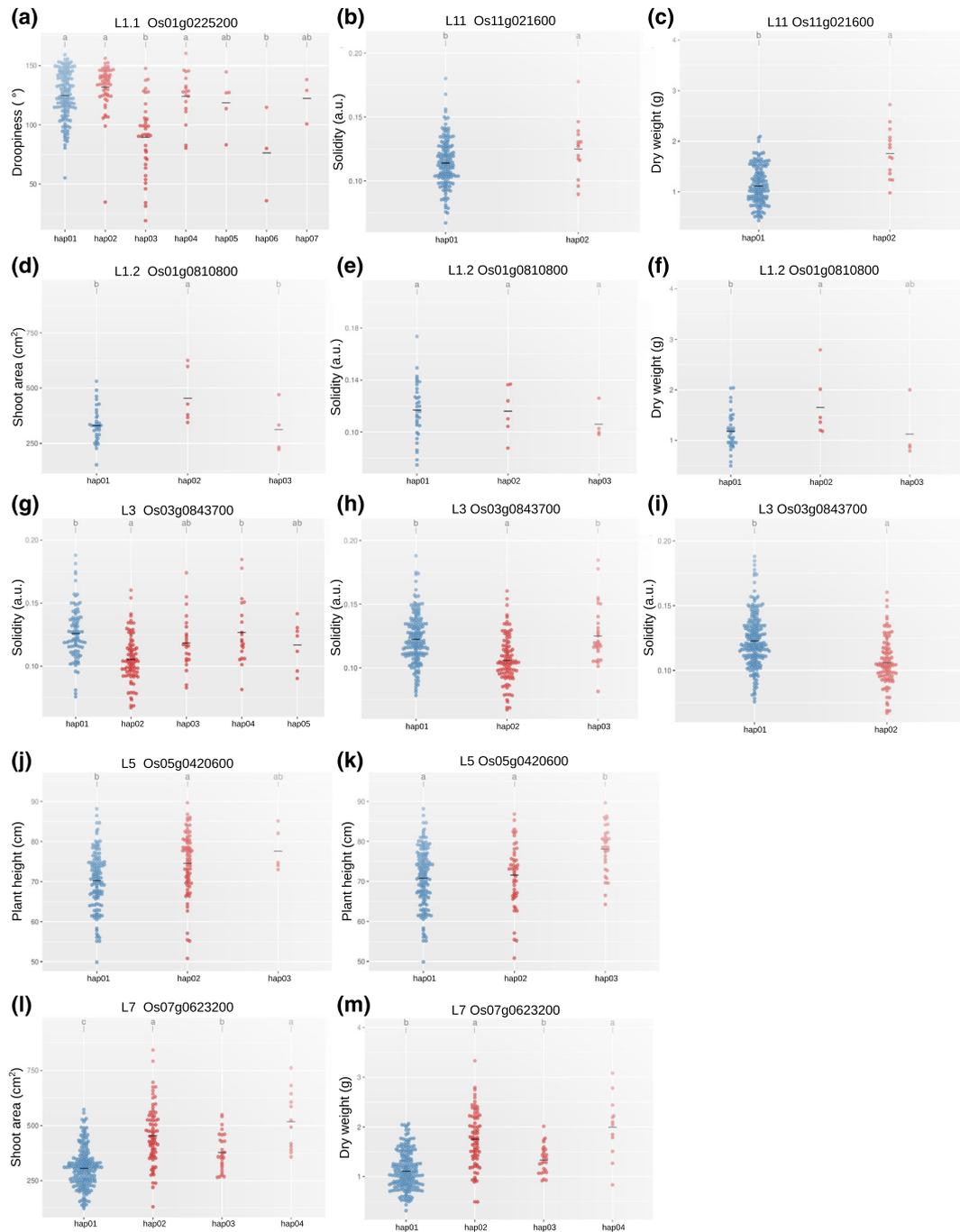


FIGURE 6 Haplotype groups and the associated effect on expression of phenotypes of core traits for shading capacity in rice. L-1.1 was detected for (a) droopiness with haplotypes in the coding sequence of the gene Os01g0225200 for a GTP binding protein. L11 was detected for (b) solidity and (c) dry weight with haplotypes in the coding sequence of the gene Os11g0216000 for a pyruvate kinase family protein. L1.2 was detected for (d) shoot area, (e) solidity and (f) dry weight with haplotypes in the coding sequence of the gene Os01g0810800 for a protein with the function of protein phosphorylation. L3 was detected for solidity, encoding the genes (g) Os03g0843700 for a FAR1 domain containing protein, (h) Os03g0845000 for a Pirin-like protein and (i) Os03g0845700 for an RPB17 fragment. L5 was found for plant height, encoding (j) Os05g0420600 for cytochrome C and (k) Os05g0420900 a conserved protein. L7 was found for (l) shoot area and (m) dry weight encoding only one gene Os07g0623200 for a heavy metal transporter protein. Dot plots with letters representing the significantly different groups, as determined with Tukey's HSD with p -value $< .05$. Y-axis trait value, x-axis groups of haplotypes, the most abundant haplotype is highlighted in blue. Additional information about the detected genes can be found in Table 4 and dot plots for haplotypes for all 13 traits found in loci of interest are shown in Dataset S7.

haploeffect_loci

Locus	gene of interest	haplotype			Effect on phenotype							
					Solidity	Shoot area	Dry weight	Number of leaves	Culm height	Droopiness	Leaf angle	Tiller angle
L-1.1	Os01g0225200 Predicted protein; BP: GTP binding	hap01	most abundant									
		hap02										
		hap03		reference								
		hap04										
		hap05										
		hap06										
		hap07										
L-1.2	Os01g0810800 Hypothetical conserved gene; BP: protein phosphorylation	hap01	most abundant	reference								
		hap02										
		hap03										
L-3	Os03g0843700 FAR1 domain containing protein	hap01	most abundant									
		hap02		reference								
		hap03										
		hap04										
		hap05										
	Os03g0845000 Pirin-like protein	hap01	most abundant									
		hap02		reference								
Os03g0845700 RPB17 fragment	hap01	most abundant										
	hap02		reference									
	hap03											
L-5	Os05g0420600 Cytochrome C	hap01	most abundant	reference								
		hap02										
		hap03										
	Os05g0420900 Conserved hypothetical protein	hap01	most abundant	reference								
		hap02										
		hap03										
L-7	Os07g0623200 Heavy metal transporter protein; ATPase	hap01	most abundant	reference								
		hap02										
		hap03										
		hap04										
L-11	Os11g0216000 Pyruvate kinase family protein	hap01	most abundant	reference								
		hap02										

FIGURE 7 Haplotype effects on all core trait phenotypes in rice. The heatmap shows for each specific haplotype within each gene of interest, an increased trait value in yellow, no significant change in grey and a decreased value in blue. The most abundant haplotype within the studied population and the haplotype comprising the reference genome are indicated. BP: biological process. Additional information about the detected genes in loci of interest can be found in Table 4.

of plant height without undesirable trade-offs with other traits. Although only a few of the detected alleles affect leaf angles, the ones that do have the opposite effect on tiller angles.

4 | DISCUSSION

We studied phenotypic and genetic variation in rice shoot architecture to identify traits and their underlying genetic loci that contribute to canopy shading. We investigated variability across a natural rice diversity panel in shoot architecture at the early vegetative stage. The traits investigated here encompass both early vigour and shade casting through shoot architecture, which are linked to weed suppression in rice fields (Andrew et al., 2015; Brainard et al., 2005; Mahajan & Chauhan, 2013; Seavers & Wright, 1999; Worthington & Reberg-Horton, 2013). It is well documented that weeds are substantially affected in their growth by shading and effectively suppressed by rice canopy shading (Chauhan, 2012, 2013; Koarai & Morita, 2003). It has also been shown that early weed suppression correlates with enhanced yield (Mahender et al., 2015; Namuco et al., 2009; Subedi et al., 2019; Zhao et al., 2006a). Traits related to shoot architecture, such as leaf angle or droopiness, are of special interest as they do not require substantial resource investment while creating more optimal 3D canopy distribution of the shoot biomass for increased shading potential. Other traits, such as leaf area, number of leaves or shoot

biomass, likely require considerable resource investments and are typically associated with growth vigour, that is, rapid seedling establishment.

4.1 | Shoot architecture traits for shading

Shading potential can be defined in two-dimensional measures, such as ground cover or projected shoot area, including a third dimension where plant height is considered as space resource utilisation (Zhang et al., 2019). We identified how all measured traits are related to one another and identified core traits that capture the observed variance (Figure 2). We identified groups of traits related to branchiness (number of leaves and tillers) and height (plant height, culm height and leaf length) and added solidity, leaf and tiller angles and droopiness to calculate a shading rank. The varieties with the highest shading rank belong to the *indica* and *aus* subpopulations, which have also been found in earlier studies to have higher yields and less weed biomass in weedy fields compared with *japonicas* (Zhao et al., 2006b). We found *admixed*, *tropical japonica* and *aus* subpopulations to typically range between *temperate japonica* and *indica*. This pattern could be found in the majority of the measured traits and is in line with the phylogenetic relatedness of the different subpopulations (Eizenga et al., 2014; Liakat Ali et al., 2011; McCouch et al., 2016; Zhao et al., 2011). Early seedling vigour is particularly important for weed competition during

the critical period of weed control, and some of the high-ranking varieties, such as Shim Balte, Paung Malaung and Sabharaj, are also known by breeders for their early vigour. Increased shading ability is intrinsic to early vigour because it follows to some extent from large size. However, the shading rank proposed here is more comprehensive to additional traits such as solidity and plant architecture that may involve less resource investment than vigour traits. With this improved way of ranking a plant's shading capacity, our study exemplifies a new method of selection for high-shading varieties and genetic loci associated with traits that contribute to high-shade canopy architecture. It also helps to narrow down any selection to a modest number of core traits, making phenotyping more efficient. We propose that varieties that have a high shading rank, are potentially more weed-competitive varieties, whereas those that rank low are likely to be weaker competitors. Indeed, our experiment proved that canopies of high-ranking varieties allow significantly less light penetration than low ranking ones (Figure 4).

The correlations between traits encapsulated within each of the trait groups that were used for the shading rank often underlines natural growth patterns; the more tillers a plant has, the more leaves it bears since each tiller has the potential to develop a certain number of leaves. A strong correlation was previously observed between tiller formation and relative growth rate (Dingkuhn et al., 2001). Likewise, in our study, the number of leaves and leaf area were positively correlated with shoot dry weight (Figure 2, Figure S1). This well-established relationship (Caton et al., 2003; Dingkuhn et al., 2001; Poorter et al., 2012) probably follows from a larger shoot area providing a higher capacity for photosynthesis and thereby leading to a higher overall growth rate (Caton et al., 2003). However, not all traits showed expected correlations; while solidity is the ratio of shoot area and hull area, it is only weakly correlated with shoot area (Figure 2, Figure S1). This suggests that shoot solidity is independent of how large its total shoot area, leaf number or angles are. Since solidity indicates the uniformity of the plant's ability to shade its circumference, it is a valuable trait for shading capacity analysis; a large projected shoot area with low solidity would still leave many open spaces within a single plant's sphere for light penetration where weeds can proliferate. Inverse correlations were found between branchiness (number of leaves and tillers) and height traits. This trade-off between height and branching is well documented as apical dominance, where the height growth of the main shoot is promoted at the expense of branching (Roig-Villanova & Martínez-García, 2016; Teichmann & Muhr, 2015). Summarising, the trends observed within this study are in line with earlier observations, whereas we identify new, informative trait groups that allow interpretations at the canopy level and that contribute independently to the shading potential of rice plants.

4.2 | Elucidating the genetic components of shading potential

We screened a large diversity panel representing different subpopulations, which adds new information to several available studies on

specific subpopulations or recombinant inbred lines (Cordero-Lara et al., 2016; Hoang et al., 2019; To et al., 2019; Wang et al., 2011).

4.2.1 | Architecture

The SNP dataset from the rice diversity panel (Eizenga et al., 2014) was combined with the observed phenotypic variation to identify putative genetic loci underlying high shading potential. This variation (Figure 1, Table S4), together with a high trait heritability (Table S5), provides a strong basis for GWAS. Plant height and leaf length were associated with loci on chromosomes 5 and 6. The locus on chromosome 5 harbours two genes encoding cytochrome C and a conserved hypothetical protein. The haplotype analysis revealed one allele for both genes that was associated with a highly significant increase in plant height (Figure 6). The locus on chromosome 6 encodes the *heading date* (*Hd1*) locus that was also previously associated with plant height in vegetative rice plants (Yang et al., 2014; Zhang et al., 2012). Subedi et al. (2019) performed a GWAS on plant height at plant maturity and found peaks on chromosomes 1 and 11. This discrepancy could indicate that at different developmental stages, plant height is determined by different genomic regions, but since Subedi et al. (2019) used a specifically constructed genetic population stemming from six parents, the genetic starting material was also fundamentally different from the population used here. Interestingly, haplotypes associated with high culm height exhibit low plant height and vice versa (Dataset S7). Haplotypes associated with high plant height typically show longer leaf length (Dataset S7). While all the height-related traits were highly correlated at the phenotypic level (Figure 2), the lack of common loci for all the traits (Dataset S4) and opposite trends within the haplotype groups (Dataset S7) suggest that the three components of plant height are regulated independently at the genetic level.

Although we consider solidity a composite trait, we revealed only one strong locus with several significant SNP associations on chromosome 3 (Figure 5). When we grouped varieties into haplotype groups for two coding regions (Os03g0845000 and Os03g0845700, Figure 6a,b), encoding a Pirin-like protein and a RPB17 fragment within this locus, the phenotypes of the haplotype groups appeared to differ not just in solidity but also in shoot area, dry weight and leaf number (Figure 6h,i, Dataset S7). This indicates that genetic regulation of solidity could still be associated with traits of plant vigour.

4.2.2 | Vigour

Vigour-related traits (i.e., dry weight, shoot area and number of leaves) are all strongly correlated and share associated loci on chromosomes 1, 7, 11 and 12 (Figure 5, Dataset S4). The locus on chromosome 11 was also reported by Yang et al. (2014) for dry weight and fresh weight at the late tillering stage, which is comparable to the developmental stage studied here. A closer look at this locus revealed that

only one gene is located within the linkage disequilibrium of associated SNPs. Interestingly, the haplotype analysis for SNPs within this gene, encoding a pyruvate kinase family protein, revealed a significant difference in dry weight between the two haplotype groups (Figure 6g). Significant differences were also observed for shoot area and the number of leaves and tillers for the same two haplotype groups. As only one gene was located within this locus and one specific haplotype was related to high biomass, this locus is a promising candidate for follow-up studies and breeding programmes. The locus on chromosome 7 associated with shoot area and dry weight (Figure 6e–f) harbours two genes, and we found that the haplotypes were associated with an increased shoot area and dry weight but also an increased number of leaves and tillers. These loci for plant vigour complement those found in a QTL study for height at 7 and 14 days after sowing and fresh weight, using exclusively *temperate japonica* genotypes (Cordero-Lara et al., 2016), thus having an intrinsically different pool of biological variation that can provide different genomic leads.

4.3 | Improving shading potential and weed suppression

The large phenotypic variation, high abundance of haplotypes that do not positively contribute to shading potential, and low shading rank of several commercially important varieties in this studied diversity panel together indicate a strong potential for improvement of shading capacity in such varieties. For example, IR 64 and Nipponbare are widely grown rice varieties that have a very low shading rank, ranking in the lowest quartile of our population (Table S3). We identified a suite of alleles of the Os01g0225200, Os01g0810800, Os03g0843700 and Os03g0845000 genes that contribute positively to shading potential (Figure 6). The IR 64 variety, which gave rise to many of the current widely grown rice varieties (Acevedo-Siaca et al., 2020; Mackill & Kush, 2018), is typically not carrying the favourable alleles for these genes, and this is true for Nipponbare as well. Remarkably, the most abundant haplotype, tends to be the most inferior one for the target traits of high shade casting, in the diversity

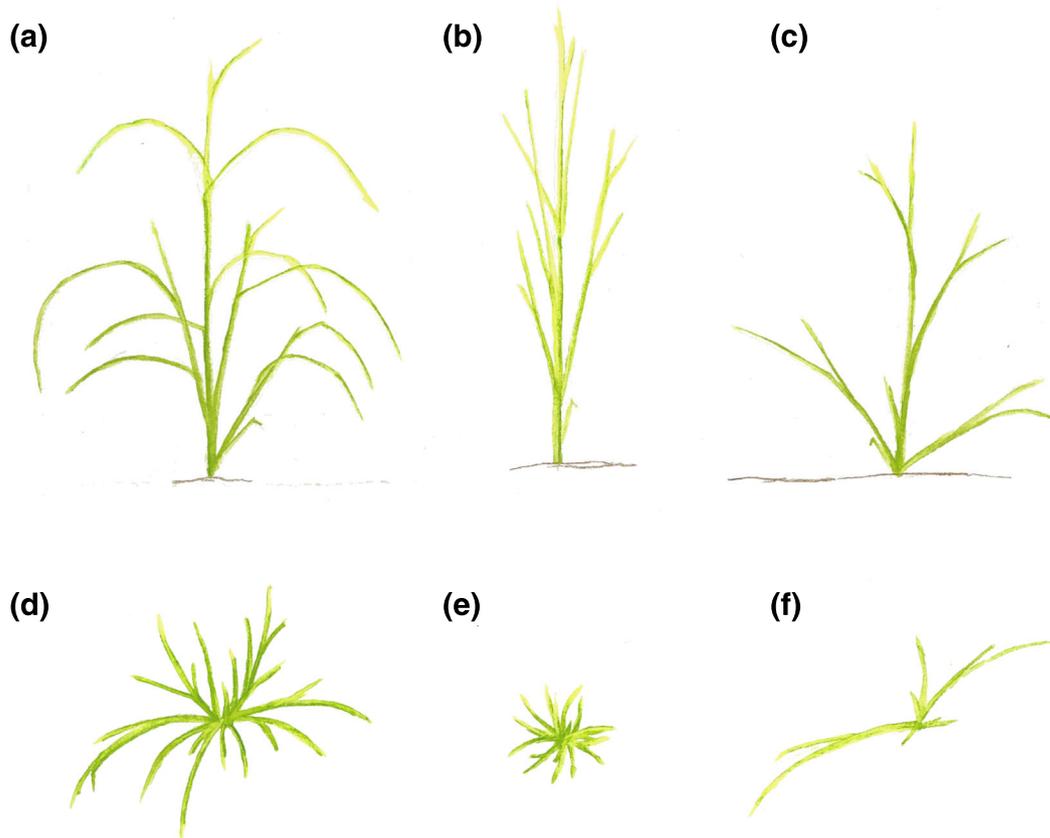


FIGURE 8 Schematic of rice plant architecture ideotypes (a,d) of high shading capacity, compared to suboptimal (b,c,e,f) shoot architecture for shading. The ideotype for a high shade casting rice plant at early growth stages, would combine strong growth vigour (many leaves and tillers and increased height) with architectural aspects, such as wide tiller and leaf angles as well as droopy leaves. This is opposed to shoots that, even if they have many leaves, have a very erect stature with narrow angles and straight up leaves (b) or a plant with long but few leaves (c). The ideotype (d) would result in a large and dense ground cover (a big shoot area, together with high solidity). A plant with very long leaves and wide inclination angles but low in vigour, would range far but only cover a small percentage of ground within this circle (low solidity) (f). The opposite, a shoot with very narrow inclination angles and upright leaves would have a high solidity but still not reach a large ground cover. Side views shown in panels a–c, top views shown in panels d–f.

panel screened here. Based on the insights from this study, we can now guide improvements for shading potential in these varieties through conventional breeding, where we provide information for optimal alleles. That a certain set of shoot architectural traits, defined here as core traits, indeed contribute to increased shading, i.e. an increased light extinction below the canopy, was confirmed in the greenhouse experiment with varieties contrasting in their Shading Rank, with high ranking varieties significantly shading more than low ranking varieties. The single metric of the Shading Rank, collapses the multidimensional description of our phenotypic screen, with the purpose of making it a more graspable and practically, easy-to-use metric for future genetic screening by scientists and breeders alike. The Shading Rank was not developed as a score for shoot architecture of any rice variety, but as a simple metric for high or low shading potential within this diversity panel. Shoot architecture traits that have been previously shown to be relevant for weed-competitiveness through light competition, are rapid leaf formation and tillering, together with wide leaf and tiller angles and leaf droopiness, leading to increased leaf area, as well as plant height (Andrew et al., 2015; Brainard et al., 2005; Dass et al., 2017; Mahajan & Chauhan, 2013; Seavers & Wright, 1999; Worthington & Reberg-Horton, 2013). An ideotype for strong shade casting ability would combine traits related to growth and vigour, together with an optimal 3D arrangement of leaves and tillers to maximise ground cover (Figure 8), leading not only to maximum shading over competitive plants but also optimising light extinction for photosynthetic activity, which in turn promotes plant growth.

Future studies could resolve if such improved varieties would indeed have superior weed-suppressive properties in field trials, as predicted from our analyses. Such tests are especially relevant because rice is a highly plastic species known for its strong ability to fill up empty spaces with tillers (Bahuguna et al., 2021). Since we have performed our experiments under stable conditions in a controlled environment, it will be relevant to perform field trials when testing improved varieties since rice varieties may differentially adapt some of the observed architecture variables under different planting densities and the associated changes in light composition and availability. Furthermore, plant architecture is also plastic throughout development, and although relatively horizontal angles would improve weed-shading early on, more erect leaves would prevent crop shading in later stages (Mantilla-Perez et al., 2020; Murchie & Burgess, 2022; Natukunda et al., 2022). Another aspect of weed competitiveness that was not covered in our study would be the root systems, for which the rapidly evolving high-throughput phenotyping methods are a major opportunity to resolve comparable questions as done here for shoot architecture. We conclude that breeding for specific vigour traits will likely have additional beneficial effects, as indicated by the haplotype studies. Vigour from root growth can then be an added layer at a later step towards field-grown, weed competitive varieties that can be farmed in a sustainable manner. Having worked from a broad diversity panel rather than a focused or limited population and including traits such as angles, droopiness and solidity has enabled us to identify alleles in existing varieties that can now be used in rice improvement programmes for sustainable weed competitiveness.

AUTHOR CONTRIBUTIONS

Martina Huber, Ronald Pierik and Rashmi Sasidharan designed the experiments, with additional input from Kaisa Kajala, Justine Toulotte and Hans van Veen. Martina Huber performed all the experiments, analysed the data and wrote the article with the contributions of all authors. Magdalena M. Julkowska carried out the haplotype analysis and assisted with statistical data analysis and data visualisation. L. Basten Snoek provided technical assistance for the genome-wide association studies (GWAS) and performed part of the analysis. Hans van Veen provided assistance for statistical analysis. Justine Toulotte performed part of the experiment and measurements. Virender Kumar contributed to the research plan and experiment support at IRRI. Ronald Pierik serves as the author responsible for contact and ensures communication, supervised all experiments, revises the manuscript draft and, together with Rashmi Sasidharan, conceives the research plan and project design.

ACKNOWLEDGEMENTS

We thank Ricardo Eugenio and James Edgane for their substantial assistance in the phenotyping at the International Rice Research Institute. Special thanks goes to Haley Schuhl and the PlanctCV team for their support and effort in tailoring the image analysis software to our data set. We thank Roel van Bezouw and Tom Theeuwen for helpful discussions about GWAS and Rens Voeselek, Evelyn Aparicio, Jochem Evers and Jonne Rodenburg for useful discussions on this research project.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest for this research.

DATA AVAILABILITY STATEMENT

All custom R scripts and supplementary data are available at <https://zenodo.org/record/6793832> or upon request to the author.

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SUPPORTING INFORMATION

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How to cite this article: Huber, M., Julkowska, M. M., Snoek, L. B., van Veen, H., Toulotte, J., Kumar, V., Kajala, K., Sasidharan, R., & Pierik, R. (2024). Towards increased shading capacity: A combined phenotypic and genetic analysis of rice shoot architecture. *Plants, People, Planet*, 6(1), 128–147. <https://doi.org/10.1002/ppp3.10419>