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Genome wide association analysis identifies candidate genes for fruit quality and yield in *Actinidia eriantha*

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Abstract

Quality and yield are the primary concerns in kiwifruit breeding, but research on the genetic mechanisms of fruit size, shape, and ascorbic acid (ASA) content is currently very limited, which restricts the development of kiwifruit molecular breeding. In this study, we obtained a total of 8.88 million highly reliable single nucleotide polymorphism (SNP) markers from 140 individuals from the natural hybrid offspring of *Actinidia eriantha* cv. 'White' using whole genome resequencing technology. A genome-wide association study was conducted on eight key agronomic traits, including single fruit weight, fruit shape, ASA content, and the number of inflorescences per branch. A total of 59 genetic loci containing potential functional genes were located, and candidate genes related to single fruit weight, fruit length, ASA content, number of inflorescences per branch and other traits were identified within the candidate interval, such as *AeWUSCHEL*, *AeCDK1* (cell cycle dependent kinase), *AeAO1* (ascorbic oxidase) and *AeCO1* (*CONSTANS-like 4*). After constructing an RNAi vector for *AeAO1* and injecting it into the fruit of cv. 'Midao 31' to interfere with the expression of the *AeAO1* gene, the results showed that the activity of ascorbic oxidase in the fruit of 'Midao 31' significantly decreased, while the content of ASA significantly increased. This study provides valuable insights into the genetic basis of variation in *A. eriantha* fruit traits, which may benefit molecular marker-assisted breeding efforts.

Keywords: *Actinidia eriantha*, GWAS, SNP, QTLs, fruit quality

1. Introduction

Actinidia eriantha is an important horticultural crop that has been widely used in kiwifruit functional genomics and hybridization introgression breeding. It is a distinctive and significant germplasm resource with a wide distribution across the vast mountainous areas of southern China. In recent years, several cultivars, including 'White' (Wu *et al.* 2009), 'Ganmi 6' (Xu *et al.* 2015) and 'Midao 31' (Wang *et al.* 2023), have been developed through the use of

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A. *eriantha* germplasm. Compared to *Actinidia chinensis*, *A*. *eriantha* exhibits several superior traits such as a high ascorbic acid (ASA) content, a relatively short juvenile phase and strong disease resistance (Wang *et al.* 2023). However, the small fruit size with low sugar content and high acid content has apparently hindered the utilization of *A*. *eriantha* in the kiwifruit industry.

Because of the dioecious nature of kiwifruit, male plants are unable to produce fruit, making traditional cross-breeding methods unpredictable. Thus, developing molecular breeding techniques in kiwifruit has become urgent and necessary. However, current knowledge about the molecular basis of important agronomic traits in kiwifruit remains very limited. Agronomic traits, such as inflorescence number, sugar content, ASA content, acid content and fruit weight, are essential for kiwifruit production and are the primary targets for genetic improvement. Despite recent progress in the characterization of functional genes associated with various kiwifruit traits, such as fruit color (Wang *et al.* 2019; Liu Y F *et al.* 2022; Wang *et al.* 2022; Shu *et al.* 2023), ASA (Liu X Y *et al.* 2022), sugar (Zhang *et al.* 2018; Han *et al.* 2023), and volatile substances (Wang *et al.* 2022), there is still much to discover regarding the mechanisms that govern fruit quality and yield formation and regulation.

Genome-wide association studies (GWAS) aim to utilize the differences in the allele frequencies of genetic variants between ancestrally similar but phenotypically diverse individuals to identify associations between the genotypes and phenotypes (Uffelmann *et al.* 2021). Initially developed in the field of human disease genetics (Ozaki *et al.* 2002), GWAS has also proven to be valuable for developing molecular breeding markers and uncovering the genetic mechanisms underlying agronomic traits in crops. In recent years, GWAS analysis has been performed for many horticultural plants, such as peach (Li *et al.* 2019), *Brassica napus* (Lu *et al.* 2019), watermelon (Guo S G *et al.* 2019), grape (Guo D L *et al.* 2019; Zhang *et al.* 2022) and pear (Zhang *et al.* 2021), to reveal the formation mechanisms of key agronomic traits. Unfortunately, progress in population studies of kiwifruit has been slow, primarily due to the difficulty of genetic population construction in kiwifruit as a perennial woody and dioecious plant. A few studies on kiwifruit have used GWAS or high-density genetic maps to locate key traits such as gender (Zhang *et al.* 2015), cuticle (Macnee *et al.* 2021), male pollen characteristics (Liao *et al.* 2021b) and fruit quality (Popowski *et al.* 2021). Research using population genetics methods to locate traits related to fruit quality and yield formation in *A*. *eriantha* has not yet been conducted.

In this study, we established a naturally-pollinated hybrid progeny population using *A*. *eriantha* cv. 'White' as the female parent, which included 140 female individuals cultivated in Hefei, Anhui Province, China. Upon an evaluation of their horticultural traits, we found that there was substantial diversity in both the number of inflorescences and fruit traits among the individuals in that population. We performed a whole-genome resequencing with an average coverage of approximately 16-fold on the 140 plants, and then conducted GWAS to identify candidate genomic regions and/or loci associated with eight agronomic traits. The results from this study will provide insights into the genetic controls of several important traits and valuable resources for molecular breeding in kiwifruit.

2. Materials and methods

2.1. Plant materials and phenotyping

A total of 140 female plants were selected from the progeny of 'White' through natural pollination. The plants were grown at the Wanzhong Experimental Station of Anhui Agricultural University, China (location: 117.23°E, 31.49°N; altitude: 9.5 m; annual precipitation: 800–1,200 mm; annual average temperature: 15.4°C), with consistent soil fertility conditions, and standard management practices.

A total of eight traits were measured, including fruit weight, fruit length, fruit width, fruit shape index, brix, acidity, ASA content, and average number of inflorescences per fruiting branch. The fruit shape index is equal to the ratio of the fruit length to the fruit width. The brix and acidity were measured using a digital hand-held refractometer (Atago, Tokyo, Japan). The determination of ASA content was carried out using the Fe^{3+} reduction method (Kampfenkel *et al.* 1995). All experiments included three replicates, with each replicate consisting of three mixed fruit flesh samples. The number of inflorescences was investigated before flowering, with 10 representative branches surveyed per tree, and the mean value was calculated. Unfortunately, many plants died due to the extreme drought in 2022, so the phenotype data were recorded for only one year in 2021.

2.2. Whole-genome resequencing and genotyping

Fresh and healthy leaves were taken after sprouting and immediately frozen in liquid nitrogen. DNA was extracted using the CTAB method (Allen *et al.* 2006), and sequencing was performed on the NovoSeq 6000 platform (150 bp paired-end reads). The low-quality reads were filtered out, and the adapters were trimmed using Fastp

software with default parameters. The filtered reads were aligned to the 'White' reference genome (Tang *et al.* 2019) using BWA-MEM with default parameters (Li and Durbin 2009). Then the duplicate reads were eliminated with the Picard package (v1.87) (http://broadinstitute.github.io/picard/). The static mapping rate was determined using samtools flagstat (Li *et al.* 2009). Finally, single nucleotide polymorphisms (SNPs) were detected using the Genome Analysis Toolkit (GATK) package (McKenna *et al.* 2010). The SNPs were filtered using MAF≥0.05 and a missing rate of no more than 10% as the criteria. The sequencing data have been deposited at Sequence Read Archive database in NCBI under accession number PRJNA995803.

2.3. GWAS

The population structure was calculated using ADMIXTURE software (Alexander *et al.* 2009) with the optimal number of ancestral components (*K* value) ranging from 2 to 4. The phylogenetic analyses were performed using Phylip software with the neighbor-joining (NJ) method. PCA was performed using PLINK (Purcell *et al.* 2007). GWAS analysis was performed using the EMMAX program (Zhou and Stephens 2012) and the TASSEL-GLM algorithm (Bradbury *et al.* 2007). The QQ plots and Manhattan plots were drawn using R software. The genes near the significant SNP locus (within 100 kb) were selected as candidate genes and annotated according to the KGD database (Yue *et al.* 2020).

2.4. RNA interference of *AeAO1* in 'Midao 31'

To create RNAi constructs of *AeAO1*-RNAi, forward and reverse PCR-amplified cDNA fragments of *AeAO1* (primers for amplification are listed in Appendix A) were inserted into the 2× CaMV35S-driven vector pHB. The RNAi constructs and the empty pHB vector were independently transformed into *Agrobacterium tumefaciens* strain GV3101. To inject the 'Midao 31' fruits (a new cultivar derived from the hybrid progenies between 'White' and 'MHX-1', which has been officially recognized by the Variety Certification Committee of the Anhui Society for Horticultural Science in 2022) at 140 d after flowering, we used an *A. tumefaciens* culture containing individual RNAi constructs or the empty vector (0.4 mL, OD_{600}=0.8), which was injected into the fruits either from the top or bottom. On the 30th d after the injection, we collected the fruits to measure the activity of the ascorbate oxidase (AO) enzyme and the content of ASA. This experiment was carried out with at least three biological replicates.

3. Results

3.1. Phenotypic variations of the 140 individuals

The variations in eight traits in 2021 were evaluated using the statistics of seven indicators: mean, maximum, minimum, standard deviation, coefficient of variation, kurtosis, and skewness (Table 1; Appendix B). The results revealed that the eight traits varied extensively among the 140 individuals (Table 1; Appendices B and C). The largest variation was in acidity (CV=29.65%), ranging from 0.56 to 2.23%, followed by fruit weight (CV=26.56%), ASA content (CV=21.22%), and inflorescence number (CV=17.08%). The smallest variations were observed in fruit width (CV=10.03%) and brix (CV=10.25%), ranging from 23.62 to 40.64 mm and from 9.68 to 16.57%, respectively. These results showed that the different fruit traits exhibited varying levels of heritability. According to the skewness and kurtosis presented in Table 1, the eight traits of the F_1 generation showed an approximately normal distribution, indicating that these eight traits are quantitative traits that follow a normal distribution. The phenotype data were recorded for only one year in 2021, owing to tree losses caused by drought damage in 2022.

3.2. Genotyping

Through whole-genome resequencing, a total of 1.61 Tb

Table 1 Descriptive statistics of the eight traits in *Actinidia eriantha*[1)]

Trait	Mean	Max	Min	SD	CV (%)	Kurtosis	Skewness
Fruit weight (g)	35.65	82.05	13.00	9.47	26.56	6.51	1.12
Fruit length (mm)	58.08	74.93	34.81	6.44	11.09	3.33	–0.29
Fruit width (mm)	30.77	40.64	23.62	3.09	10.03	3.79	0.56
Fruit shape index	1.90	2.54	1.41	0.22	11.78	3.18	0.10
ASA content (mg 100 g^{-1} FW)	678.86	1,057.83	398.27	144.04	21.22	2.51	0.34
Acidity (%)	1.28	2.53	0.56	0.38	29.65	3.05	0.61
Brix (%)	13.26	16.57	9.68	1.36	10.25	3.07	–0.04
Inflorescence number	4.45	6.80	2.50	0.76	17.08	3.52	0.38

[1)] SD, standard deviation; CV, coefficient of variation.

of raw sequence data were obtained (Appendix D). After removing low-quality and adapter sequences, an average of approximately 16-fold coverage data were obtained for each accession and used for SNP calling (Appendix D). The average mapping rate of the cleaned reads to the reference genome of 'White' was 98.92%. A total of 8.88 million high-quality SNPs were obtained and used for downstream analyses. It is apparent that these SNPs were relatively evenly distributed across the 'White' chromosomes (Fig. 1). The highest number of associated SNPs was located on Chr08, whereas the lowest number was observed on Chr04 (Appendix E). The average marker density was approximately 69 bp per SNP, and Chr28 had the highest density of SNP markers (61.69 bp/SNP), while Chr04 had the lowest marker density overall (74.46 bp/SNP). The SNP density obtained in this study was remarkably high, laying a solid foundation for subsequent QTL mapping and molecular marker development.

3.3. Population structure and evolutionary analysis

Using these high-quality SNPs, we initially inferred the phylogenetic relationships of the kiwifruit and found no clear subgroup differentiation among the 140 individuals (Fig. 2-A). The PCA results indicate that all samples are dispersed on the coordinate axis, which supports the conclusion that there is no significant subgroup differentiation within the population (Fig. 2-B). To better understand the genetic structure of the 140 genotypes, we employed the ADMIXTURE software (Alexander *et al.* 2009) to analyze the SNP dataset. The optimal number of ancestral components (k) was estimated using the cross-validation technique, which involved varying numbers of subpopulations from 2 to 4. We determined that the optimal number of groups was 2 (Fig. 2-C). These findings indicated that the 140 individuals could be classified into two distinct groups: Group I and Group II, including 66 and 74 individuals, respectively. These analyses demonstrated that the tested population could be used for GWAS analysis.

3.4. Genome-wide association analysis

To further establish the association between the SNPs and agronomic traits in the 140 individuals, we used the EMMAX (Zhou *et al.* 2012) and TASSEL-GLM (Bradbury *et al.* 2007) algorithms for association analysis. By comparing the QQ plots of the two algorithms (Appendices F and G), we found that the EMMAX algorithm generated lower false-positives, making it more suitable for the population in this study. Due to the limitation of population size, we did not obtain signals exceeding the Bonferroni significance threshold ($P<5.62\times10^{-9}$). Considering that

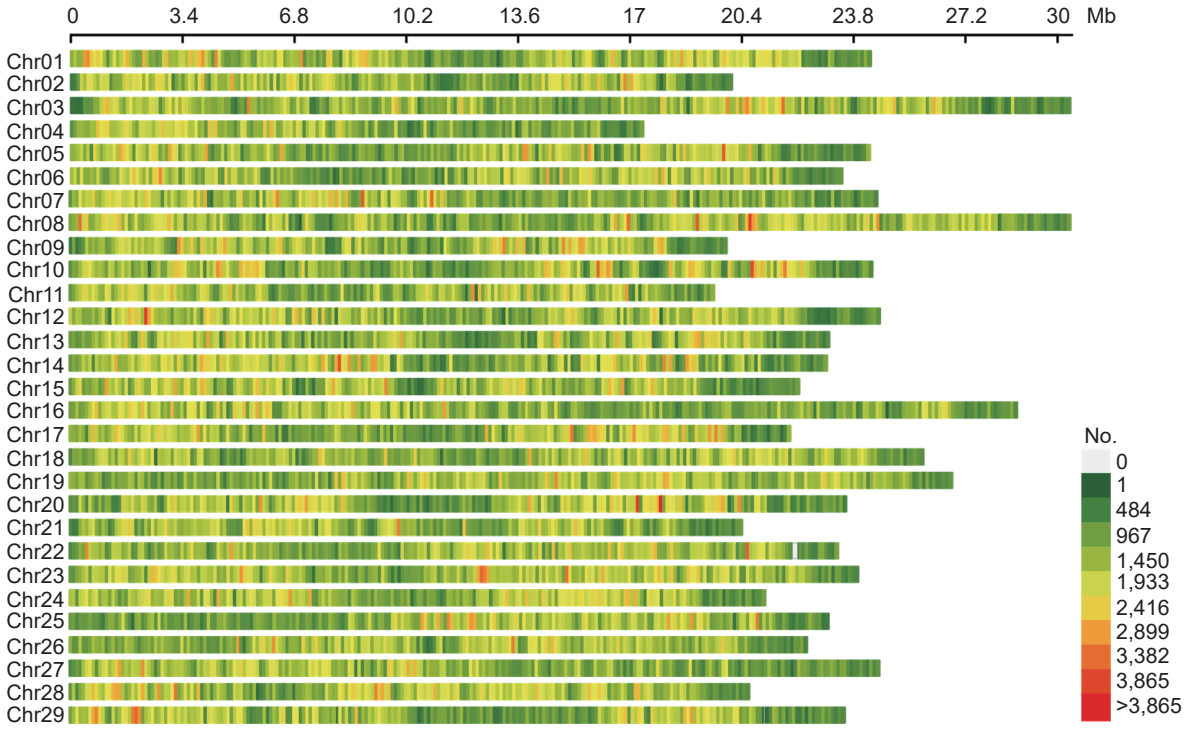

Fig. 1 Single nucleotide polymorphism (SNP) distributions on the 29 chromosomes of *Actinidia eriantha*. The window size is 0.1 Mb.

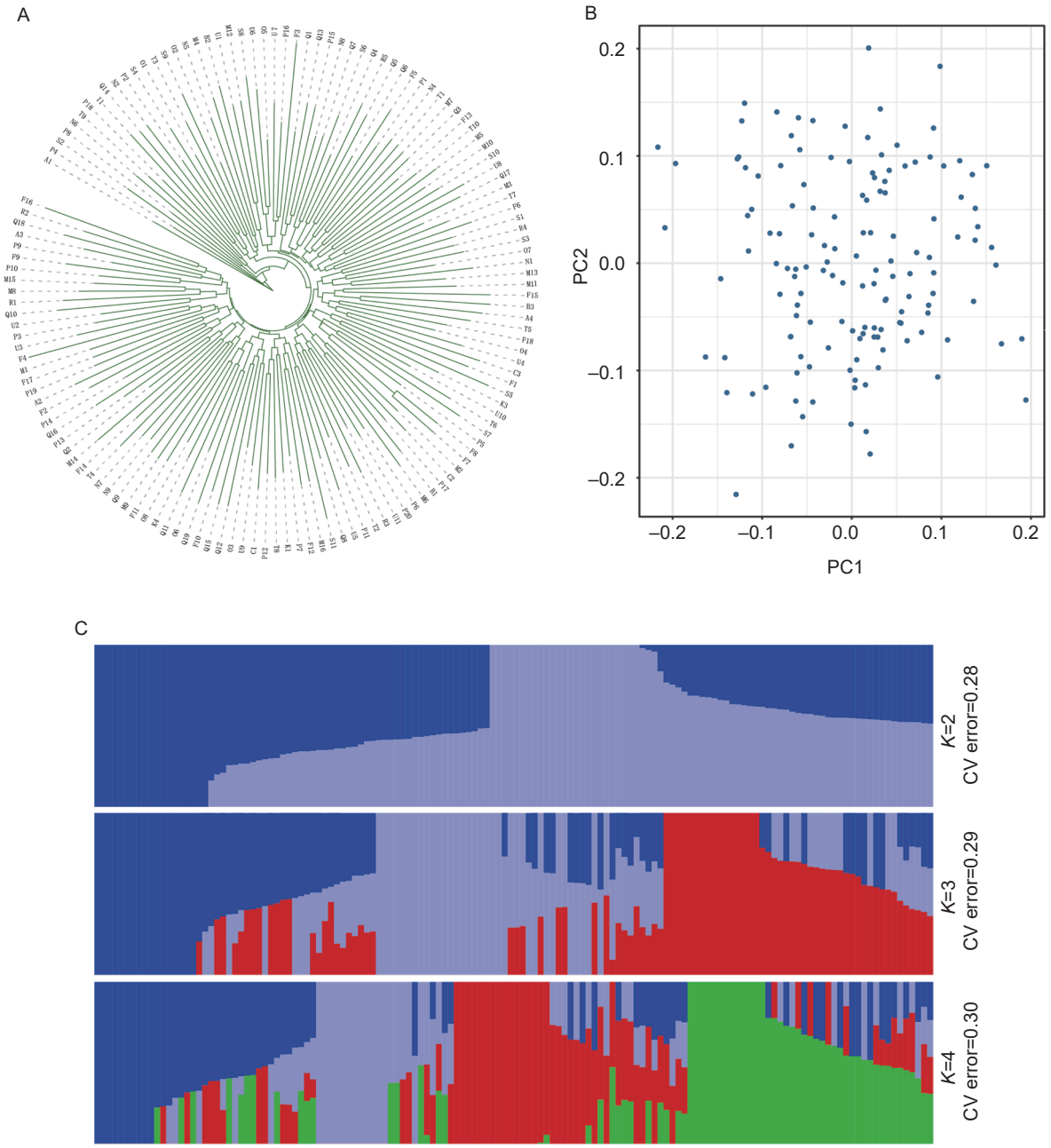

Fig. 2 Phylogenetic and population structure analysis. A, phylogenetic tree of the 140 individuals. B, principal component (PC) analysis. C, population structure analysis for *K*=2–4. CV error indicates cross-validation error.

the Bonferroni significance threshold was too conservative because it assumes that all tests are independent (Asif *et al.* 2021), we opted to establish a secondary GWAS signal threshold at a slightly lower level (fruit weight: $P<1\times10^{-7}$; other traits: $P<1\times10^{-6}$). A total of 59 candidate loci associated with the eight traits were identified (Appendix H), and subsequent analyses focused on the genes located within a 100 kb upstream and downstream interval of these SNPs, referring to previous studies (Guo *et al.* 2020; Nishio *et al.* 2021; Gong *et al.* 2022).

Fruit weight and shape are important traits that affect the yield and quality of kiwifruit. Our study revealed five SNPs that surpassed the threshold (Appendix H), with three of them located on chromosome 7 (Chr07: 16,400,877, Chr07: 16,400,887, and Chr07: 16,418,650), one on chromosome 8 (Chr08: 22,628,815), and one on chromosome 21 (Chr21: 18,682,722). A total of 29 putative coding genes were included within the candidate intervals associated with these five significant SNPs (Appendix I). Previous studies have shown that

the determination of fruit size involves several molecular mechanisms or regulatory pathways, such as hormonal regulation (including IAA, GA, CK, ABA, ETH, and BRs), the CLV-WUS signaling pathway, the ubiquitin-proteasome pathway, microRNA regulation, and endoreduplication (Zhao *et al.* 2021). Based on the gene annotations and previous characterizations of the regulation of fruit size (Muños *et al.* 2011; van der Knaap *et al.* 2014), a *WUSCHEL-LIKE* homeobox gene (*DTZ79_21g12670*) was identified as the candidate locus underlying fruit weight variation within the mapping interval 56.536 kb downstream of the linked SNP (Chr21:18,682,722) (Fig. 3-A; Appendix I). Therefore, we speculate that this locus may have a close association with the fruit weight of kiwifruit.

Compared to the variation in fruit length, the variation in fruit width was relatively small (Table 1), so the longitudinal diameter is a major factor influencing fruit shape in kiwifruit. For this trait, we found five SNPs exceeding the threshold (Fig. 3-B; Appendix H); one is located on chromosome 10 (Chr10: 12,371,117), two are located on chromosome 16 (Chr16: 26,280,282 and Chr16: 26,280,283), and two are located on chromosome 25 (Chr25: 13,131,253 and Chr25: 13,131,255). A total of 21 coding genes were identified within the candidate intervals (Appendix I), including a gene putatively encoding cyclin-dependent kinase (CDK) (*DTZ79_25g04440*) that has been reported to be associated with cell size distribution in tomato (Czerednik *et al.* 2015; Zheng *et al.* 2022). Consistently, this gene was also identified in the GWAS analysis using the fruit shape index (Fig. 3-C), indicating that *DTZ79_25g04440* may affect fruit length and shape by influencing the direction of cell division during fruit development.

Actinidia eriantha is valued by researchers and consumers for its high ASA content, but the molecular basis of its ASA metabolism remains poorly understood. In this study, we discovered a total of 11 significant SNPs associated with ASA content, and all 11 SNPs were located on chromosome 28 (Fig. 3-D; Appendix H). We identified a gene putatively encoding ascorbate oxidase (*AeAO1/DTZ79_28g06800*) (Fig. 3-D; Appendix I), which plays an important role in the recycling of ASA, located in the downstream 20.275 kb interval of a candidate locus (Chr28: 11,146,558). *Agrobacterium*-mediated transient transformation was used to introduce an *AeAO1*-RNAi vector into the developing fruit of 'Midao 31'. The results showed that in *AeAO1*-RNAi fruits, the relative expression of *AeAO1* and the activity of the AO enzyme were significantly reduced compared to the controls, and consequently the ASA content was increased (Fig. 4).

The number of inflorescences on each branch is an important trait of kiwifruit that determines its fruiting ability and yield. In this study, we identified a total of 16 SNPs associated with the inflorescence number (Fig. 3-E; Appendix H). Among these SNPs, one is located on Chr02, one on Chr05, two on Chr20, and 12 on Chr25. Within the candidate interval, we identified a gene, *AeCO1* (*DTZ79_20g15030*), putatively encoding CONSTANS-like 4 protein (Fig. 3-E; Appendix I), which is a master transcription factor in the photoperiod floral pathway that integrates upstream signals and activates the florigen gene *FLOWERING LOCUS T* (FT) in *Arabidopsis* (Lv *et al.* 2021). Thus, we assume that this gene may be associated with the number of single branch inflorescences in kiwifruit.

Notably, other than *WUSCHEL*, several other candidate genes including *AeCDK1*, *AeAO1* and *AeCO1* were also identified using the TASSEL-GLM algorithm (Appendix J). In addition, we identified two SNPs associated with fruit width and 13 SNPs associated with acidity, as well as four SNPs associated with brix content (Fig. 3-F, G, H; Appendix H). However, within these intervals, no characterized genes were found to be directly associated with these traits (Appendix I). Further expansion of the population size and integration of multi-omics data would be needed for the fine mapping and identification of the genes directly associated with these traits.

4. Discussion

GWAS is a powerful approach that utilizes natural populations to explore the genetic foundations of complex traits, and it has been widely applied in gene mapping related to important agronomic traits. The quality and yield of fruits are the primary traits that breeders prioritize. Investigating the molecular mechanisms associated with these essential traits holds great significance for both the development of new varieties and the growth of the industry as a whole. The type of population is an important factor that affects the effectiveness of GWAS. In general, natural populations are usually used for conducting GWAS, while familial populations are typically used to construct genetic linkage maps for locating key QTLs. Because natural populations undergo multiple generations of recombination, they possess smaller linkage blocks, which enables more precise localization of functional genes. However, several studies have used familial populations to conduct GWAS analyses for different crops, such as hexaploid chrysanthemum (Sumitomo *et al.* 2022), tomato (Bineau *et al.* 2021), maize (Zhang *et al.* 2019), and cotton (Jin *et al.* 2023). In addition, the population size also influences the effectiveness of GWAS. In this study, due to the limitation

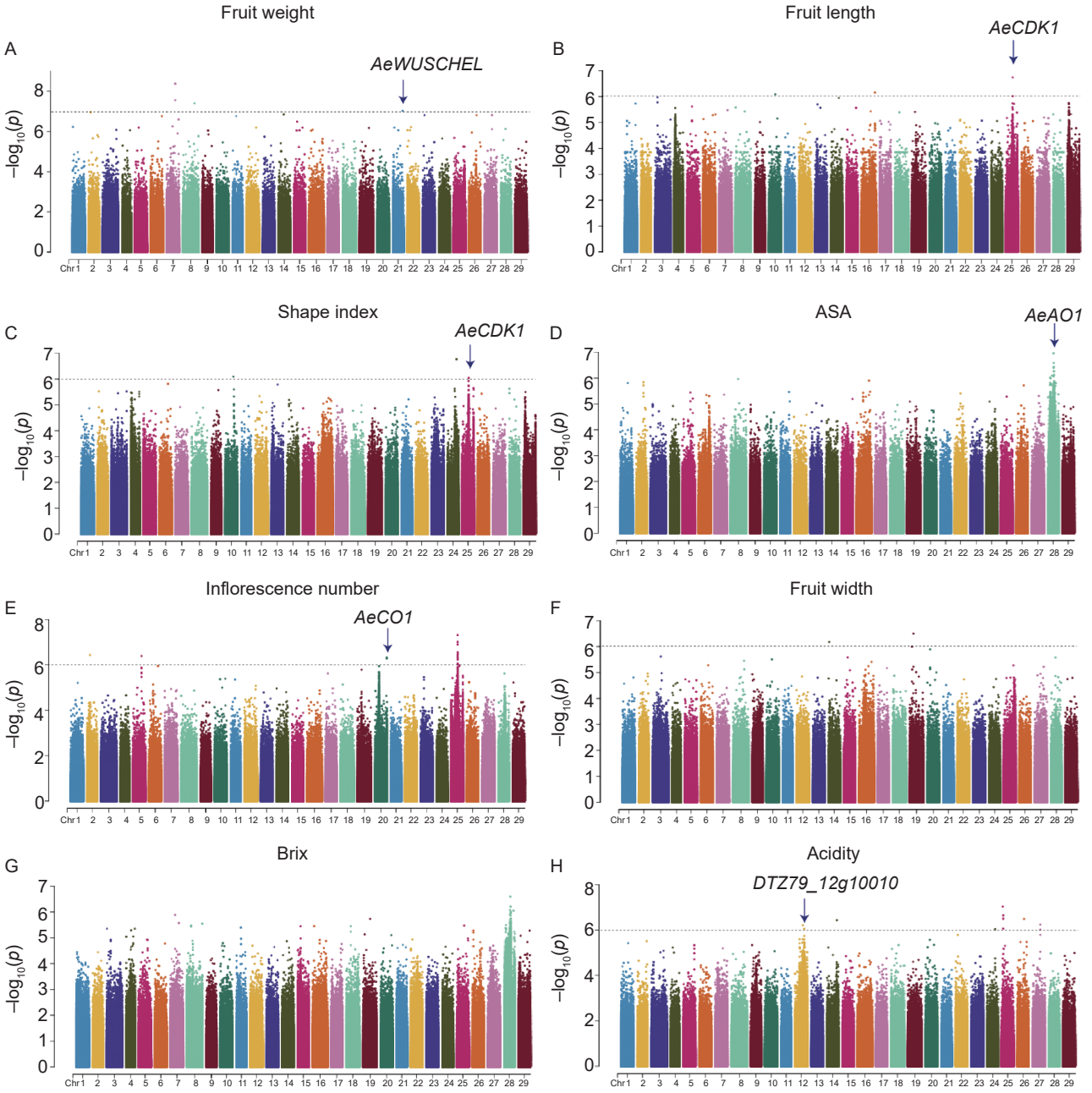

Fig. 3 Manhattan plot of genome-wide association studies (GWAS) (EMMAX) for eight traits in *Actinidia eriantha*. A, fruit weight. B, fruit length. C, fruit shape index. D, ascorbic acid (ASA) content. E, inflorescence number. F, fruit width. G, brix. H, acidity.

of the population size, we did not obtain any SNP that surpassed the Bonferroni significance threshold, so we lowered the threshold and successfully anchored the mapping signals and candidate genes associated with the phenotype. This compromised approach has also been employed in several previous studies (Li *et al.* 2019; Zhang *et al.* 2021). In this study, we collected fruit seeds from cultivated 'White' plants pollinated by unknown *A. eriantha* pollinators and generated a 'White' progeny population consisting of 140 individuals. As the pollination in the population was natural and the genotypes of the paternal parents were uncertain, we chose genome-wide association analysis to locate the eight traits related to yield and fruit quality. The results of the PCA based on SNP showed that there were no clearly defined clusters in the principal component plot, indicating that the genotypes used did not represent a highly structured population and were suitable for GWAS. Large phenotypic variations were observed for individual traits, suggesting the existence of a diverse genetic pool. This

genetic diversity provided an opportunity to explore the novel QTLs related to the fruit traits pursued by kiwifruit breeding programs. In this study, we observed significant variations in fruit weight, ASA content, and acidity within the population, indicating that these traits will have considerable potential for selection in breeding programs. Marker density is another important factor that affects the effectiveness of GWAS. In this study, we used whole-genome resequencing technology to obtain 8.88 million high-density SNP markers, which is a significantly larger number than in previous studies (Zhang *et al.* 2015; Liao *et al.* 2021b; Macnee *et al.* 2021; Popowski *et al.* 2021). Such a large number is a useful prerequisite for QTL mapping and marker-assisted breeding to facilitate the identification of new desirable traits.

Previous studies have shown that changes in cell division number and the number of carpels are the main factors that affect fruit size and shape. Several genes related to fruit size and shape have been cloned and functionally characterized, such as *ENO* (Yuste-Lisbona *et al.* 2020), *CNR* (*fw2.2*) (Frary *et al.* 2000), *KLUH* (*fw3.2*) (Anastasiou *et al.* 2007), *WUSCHEL* (Muños *et al.* 2011), *YABBY2* (Cong *et al.* 2008), *OVATE* (Liu *et al.* 2002), and

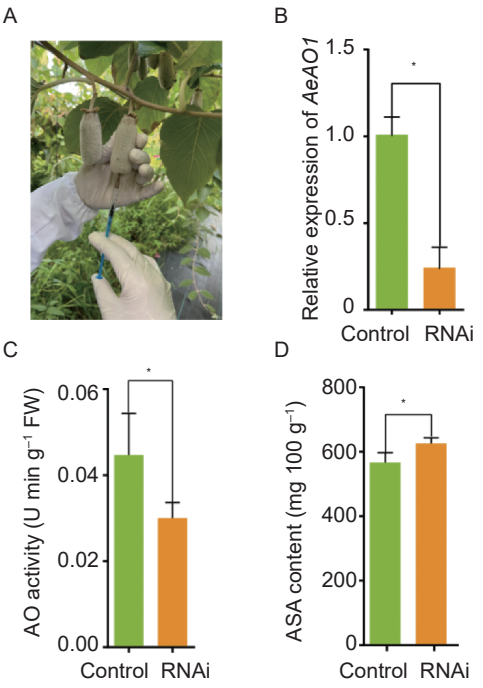

Fig. 4 Functional identification of the *AeAO1* gene in RNAi plants of *Actinidia eriantha* cultivar 'Midao 31'. A, performance of the transient expression assay in on-tree fruits of *A. eriantha* at 140 d after flowering (DAF). B, relative expression levels of *AeAO1*. C, activity of ascorbate oxidase (AO). FW, flesh weight. D, ascorbic acid (ASA) content. *Agrobacterium tumefaciens* strain GV3101 harboring 35S::*AeAO1*-RNAi was injected into fruit flesh at 140 DAF. An empty vector (pHB) was used as a control. Error bars indicate SD (n=3). *, P<0.05.

SUN (Xiao *et al.* 2008). The *WUSCHEL* gene encodes a homeodomain transcription factor that plays a crucial role in maintaining stem cell identity in the shoot apical meristem (SAM). It is essential for regulating the size of the stem cell population in all meristems, and it has a significant impact on the first phase of fruit development, which determines the final dimension of the tomato fruit (van der Knaap *et al.* 2014). Our study suggested that a *WUSCHEL-LIKE* homeobox gene located on Chr21 might be associated with fruit size control in kiwifruit. Consistently, a fruit size-related QTL was also located on Chr21 by using an interspecific population derived from hybrids between *Actinidia rufa* and *A. chinensis* (Liu 2016).

Cyclin-dependent kinase (CDK) is an A-type cyclin-dependent kinase, and an important cell cycle regulator that is conserved throughout all eukaryotes. In this study, we identified a gene annotated as CDK that might be related to the length and fruit shape index of kiwifruit in the population. A previous study showed that the cell size distribution of tomato fruit can be changed by the overexpression of *CDKA1* (Czerednik *et al.* 2015). Another study showed that knockout of the suppressor *SlMYB3R3* leads to elongated fruit, which results from an altered cell division pattern at the ovary stage by regulating cell-cycle-related genes including *CDK* and *CYCB2* (Cyclin B2) (Zheng *et al.* 2022).

The metabolism of ascorbic acid in higher plants is mainly regulated through the three pathways of biosynthesis, cycling, and degradation, with the cycling pathway playing an important role in both the accumulation and balance of ASA content in plants. L-ascorbate oxidase (AO) and L-ascorbate peroxidase (APX) are essential enzymes in the antioxidant system of plants, and they also play a critical role in scavenging free radicals (Liao *et al.* 2021a). This study identified a gene annotated as plant L-ascorbate oxidase underlying the variation in the ASA content in the population. The RNAi repression experiment showed that when the *AeAO1* gene was disrupted the activity of the AO enzyme was reduced, accompanied by a slight increase in the content of ASA. Consistently, the overexpression or pTRV2:*AcAO1*-mediated VIGS of *AcAO1* in fruits of *A. chinensis* at 120 d after flowering resulted in a reduction or an increase in the contents of reduced AsA and total AsA (Shu *et al.* 2023). The sequence alignment indicated that *AcAO1* and *AeAO1* are highly homologous (Appendix K), suggesting that *AeAO1* may possess a similar function as *AcAO1*.

Kiwifruit has mixed flower buds. They first sprout into shoot tips, and then form flower buds in several leaf axils in the lower part of the new shoots, which then bloom and bear fruit. In the population constructed in this study, we observed a very interesting phenomenon: there were

differences in the number of inflorescences on the new shoots of different individuals, with a minimum of four and a maximum of 10. In a sense, a greater number of flower clusters represents a stronger ability to set fruit and a higher yield. Our analysis suggests that the most likely gene related to flower cluster number within the candidate interval is an annotated *CONSTANS-like 4* gene, which is an evolutionarily conserved central component of the genetic pathway that controls the onset of flowering in response to daylength (Tiwari *et al.* 2010).

A recent study reported an *AcNAC1* gene (GenBank: KF319050.1) that regulates the citrate transporter, further influencing the acidity of kiwifruit (Fu *et al.* 2023). In *A. eriantha*, we identified the corresponding homologous gene DTZ79_12g10010 of *AcNAC1*. Interestingly, among the SNPs identified in this study that are associated with acidity, one SNP is located approximately 269 kb downstream of this gene. Unfortunately, there are numerous candidate genes within the candidate intervals of brix, acidity, and fruit width, so it is difficult to determine whether these genes are direct candidates related to the corresponding traits based only on annotation information.

Notably, the traits of fruit quality and yield are complex, and we need to combine different approaches for the candidate genes with retrograde validation. Specifically, in the next step, we will conduct further research to verify the functions of the candidate genes and construct a larger population size for validation experiments to further verify their possible functions in kiwifruit. Overall, this study provides valuable genomic resources and potential molecular markers for gene discovery and genetic improvement in terms of fruit quality and yield in kiwifruit.

5. Conclusion

In this study, we used the GWAS method to identify candidate genes associated with important agronomic traits, such as yield potential, fruit weight, shape, and ASA content, in 140 individuals derived from *A. eriantha* cv. 'White'. These genes provide novel insights into the genetic foundations underlying the formation of yield and quality for *A. eriantha* cultivation.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendices associated with this paper are available on https://doi.org/10.1016/j.jia.2023.11.025

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