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Detecting the genetic variants associated with key culinary traits in *Dioscorea alata*

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ABSTRACT

Quality attributes play a pivotal role in determining consumers' acceptance and market value of food crops. *Dioscorea alata* is a major yam species for food security in tropical areas, but our understanding of the genetic factors underlying tuber culinary traits is limited. This study aimed at elucidating the genetic basis of key culinary attributes, including apparent dry matter content (DM), cooking time, boiled yam hardness, and moldability, through genome-wide association studies (GWAS). Phenotypic assessment revealed notable variations among the *D. alata* genotypes and significant correlations among the quality traits. The GWAS identified 25 significant associations distributed across 14 chromosomes. A total of 12, 1, 6, 6 single nucleotide polymorphisms were detected for cooking time, moldability, hardness and DM. Allele segregation analysis of the identified loci highlighted favorable alleles for short cooking time, good moldability, high hardness and DM content. Within a set of 42 putative candidate genes, we identified genes differentially expressed in tubers of genotypes with contrasting quality attributes. Our study offers valuable insights into the links between these key culinary traits and the underlying genetic basis in *D. alata*. These findings have practical implications for breeding programs aimed at enhancing the quality attributes of greater yam.

1. Introduction

Yams (*Dioscorea* spp.) are sources of essential carbohydrates in various regions of the world, holding cultural and economic importance, particularly in West Africa (Arnau et al., 2010, pp. 127–148; Obidiegwu, Lyons, & Chilaka, 2020; Owusu Danquah et al., 2022). While a few Dioscorea species are used for food and industrial purposes, the most extensively cultivated ones include *D. alata*, *D. cayennensis*, and *D. rotundata* (Degras, 1993). *Dioscorea rotundata* is indigenous to West Africa and has the largest production volume, while *D. alata* was introduced to Africa from Asia and is the most commonly grown species globally (Sharif et al., 2020). Traditional yam breeding has long overlooked quality traits, as phenotypic evaluation of these complex properties is slow and labor-intensive (Darkwa, Olasanmi, Asiedu, & Asfaw, 2020). However, emerging tools now empower more efficient dissection of the culinary quality (https://rtbfoods.cirad.fr/). Recent advancements in near-infrared spectroscopy have shown promise in rapidly predicting quality traits such as texture and biochemical composition in yams, which can significantly accelerate breeding programs and enhance food security (Alamu et al., 2021; Ehounou et al., 2021; Houngbo et al., 2024).

Cooking quality is a crucial aspect of yam acceptance by consumers (Brunnschweiler, 2004; Tortoe et al., 2014). Traits like hardness and moldability of cooked yam (Fig. 1) are essential factors considered by

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consumers and processors when selecting yam varieties for cooking and processing purposes (Honfozo et al., 2021; Otegbayo et al., 2023). Hardness refers to the firmness or resistance of the cooked yam flesh. It is an important quality attribute as it affects the sensory experience and ease of consumption (Alamu et al., 2022; Medoua, Mbome, Agbor-Egbe, & Mbofung, 2005). Hardness is influenced by factors such as the composition of cell walls, starch structure, and secondary metabolites (Li et al., 2024). For instance, a higher proportion of amylose, a starch component, is associated with increased hardness in sweet potato (Lai et al., 2016). On the other hand, softer yam varieties typically have a higher moisture content and lower starch content. The cooking method, such as boiling or steaming, also affects the final hardness of yam (Honfozo et al., 2021).

Moldability, or poundability, refers to the ability of cooked yam to be pounded or mashed into a desired consistency (Otegbayo et al., 2023). It is a desirable trait for traditional dishes like pounded yam in West Africa, where yam is traditionally pounded with a mortar and pestle (Otegbayo et al., 2007). Cassava varieties with higher starch content, particularly varieties with a higher proportion of amylopectin, tend to exhibit better moldability due to the retrogradation of starch (Awoyale, Olatoye, & Maziya-Dixon, 2023).

Cooking time is an important quality trait in yam (*Dioscorea* spp.), influencing consumer's acceptance (Otegbayo et al., 2021). Depending on species and variety, it can range from 10 min to over an hour, while short cooking time is preferred (Otegbayo et al., 2021). Although the correlation between cooking time and dry matter content has not been established for yam, in cassava (*Manihot esculenta*)), data tend to show a positive correlation between them (Safo-Kantanka & Owusu-Nipah, 1992; Tran et al., 2021). Based on these reports, it can be assumed that dry matter content, representing starch and fiber levels, is positively associated with longer cooking time.

Efforts to enhance the cooking quality of yam, particularly hardness and moldability, in breeding programs involve genetic studies (Arnau et al., 2023; Asfaw et al., 2023; Ehounou et al., 2022; Gatarira et al., 2020). *Dioscorea alata* is renowned for its favorable agronomic performance and widespread cultivation. It exhibits a stable yield, making it well-suited for addressing the challenges posed by the current climate change scenario (DIBYab et al., 2009; Frossard et al., 2017; Hgaza et al., 2012; Kouakou et al., 2023). However, one significant drawback of this crop is its relatively low culinary quality (Adesokan, Alamu, Fawole, Asfaw, & Maziya-Dixon, 2024). In contrast, *D. rotundata* and *D. cayenensis* are characterized by superior culinary attributes. Unfortunately, interbreeding between these species is challenging due to cross-incompatibility (Mondo et al., 2022). Within *D. alata*, a diversity in culinary quality has been observed (Ehounou et al., 2021; Ehounou et al., 2022). Consequently, further genetic investigations focusing on the underlying genetic factors governing culinary quality traits in *D. alata* are necessary.

The availability of a high-quality genome sequence for *D. alata* has opened a new avenue for genetic and genomic studies of tuber quality traits (Bredeson et al., 2022). Transcriptome profiling, quantitative trait locus (QTL) mapping, and genome-wide association studies (GWAS) have identified genomic regions and candidate genes associated with various yam quality attributes, such as tuber color, texture, dry matter content, and starch accumulation (Arnau et al., 2023; Dossa et al., 2023; Mota et al., 2024; Wang et al., 2024). These findings pave the way for future efforts in genetic engineering and breeding programs to develop superior yam varieties with improved nutritional and sensory qualities. Integrating genomics and breeding approaches is crucial for enhancing yam production, ensuring higher yields, better taste, and increased nutritional value, ultimately benefiting communities worldwide that rely on yam as a staple crop.

In this study, the main objective was to unravel the genetic basis underlying the culinary quality parameters, including cooking time, dry matter content of fresh yam, hardness, and moldability of cooked one in *D. alata*. To achieve this, a diverse panel of *D. alata* accessions was carefully selected, and these accessions were evaluated for the desired traits at two locations. The combination of GWAS and gene expression profiling was employed to identify the specific genomic regions and potential genes associated with the culinary quality traits.

2. Materials and method

2.1. Plant material and experimental design

The plant material utilized in this study consisted of a diverse panel of *D. alata* genotypes (Dossa et al., 2023). The panel comprised 52 diploid genotypes collected from various tropical regions, including Africa, the Caribbean, and the Pacific. The field trials were fully detailed in Dossa et al. (Dossa et al., 2023). The genotypes were planted at two locations (Godet and Roujol) in Guadeloupe (Dossa et al., 2023).

2.2. Assessment of cooking time

Following the field harvest of the 52 genotypes, three tubers per genotype (biological replicates) per planting site were selected. To cook the tubers, three identical pressure cookers (Moulinex Inicio xl, 3 L, 1000 W; https://www.moulinex.fr/p/inicio-2-15c/8000035882) and basic kitchen utensils were employed. The method described by Ehounou et al. (Ehounou et al., 2021) was followed. First, the head and the tail were removed. Then, the tubers were peeled and sliced (~1 cm thickness). To ensure a sufficiently large water-to-sample ratio to avoid bias due to the variability in the slices' diameters and the sample quantity, an excess volume of 1.5 L of water was initially added to an oversized pressure cooker of 3 L capacity.

Five slices per tuber were then cooked in each pressure cooker until they reached the desired level of softness, determined by the fork test. This test is semi-empirical and relies on a trained operator carefully probing the surface of the slices with a fork and visually assessing their appearance, until they become soft. In similar condition, this test was

Fig. 1. Photographs of boiled and pounded *D. alata* tuber. A) Tubers of various genotypes were boiled, and different hardness levels could be observed; The blue arrow shows an example of a genotype with a low hardness, while the red arrow shows a variety with a high hardness; **B)** A genotype with a bad moldability; **C)** A genotype with a good moldability. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

estimated to give a precision between 1 and 2 min, and an accuracy of ±5 min (Tran et al., 2021). The cooking time (min) was recorded subsequently for each tuber as the point at which at least three slices out of five are considered to be cooked. This analysis was performed on *D. alata* samples from Godet and Roujol.

2.3. Assessment of moldability

Moldability is the ability of the dough from the pounded yam to form a ball. Three mechanical pounders (Robot multifonction Moulinex Double Force Compact, 800 W, 2.2 L; https://www.moulinex.fr/p/double-force-compact-blanc/8000035956) were used for this experiment following the descriptions from Ehounou et al. (Ehounou et al., 2021). Although the dough obtained from a mechanical pounder is not exactly the same as the one obtained with the traditional practice of manual kneading in a mortar with a pestle, our method ensures uniform conditions for sample preparation and guarantees the repeatability of the experiment. Once the boiled yams were ready, 10 slices from each tuber were randomly selected and inserted into the mechanical pounders. Each pounding lasts for 2 min, and the dough was assessed for its moldability quality based on a score within 0–9, with 0 meaning no ball could be formed in hand while 9 represents a ball is perfectly formed, and the overall texture is very similar to the best-pounded yam obtained from the traditional practice. This analysis was performed on *D. alata* samples from Godet and Roujol.

2.4. Assessment of apparent dry matter content

In addition to the cooking process, the remaining portion of the *D. alata* tubers that were not used for cooking underwent further analysis. In order to compare our results with previous studies, the apparent dry matter was calculated (Alamu et al., 2022; Ehounou et al., 2021; Houngbo et al., 2024; Lebot & Malapa, 2013). Samples were cut into small pieces, weighed, and then dried in an Air Convectional Oven (Memmert UN 55, GmbH) for 72 h at a temperature of 60 °C. After drying, the samples were weighed again to determine their dry weight. The apparent dry matter content of the samples was calculated using the following formula:

Apparent dry matter content (%) = (Dry weight/Fresh weight of sample) x 100.

The apparent dry matter content is expressed as a percentage and provides valuable information about the proportion of solid matter in the yam samples after moisture removal through drying. A preliminary study on more than 400 samples of *D. alata* tubers that had undergone the same preparation, estimated the average residual water content at 6.74% with a standard deviation of 2.50%. This analysis was performed on *D. alata* samples from Godet and Roujol.

2.5. Assessment of hardness

To estimate the hardness, Texture Profile Analysis (TPA) was conducted in this study as previously implemented by Mota et al. (Mota et al., 2024). The TPA measurements were performed using the TAX-T2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK). For the double-compression test performed, two compression cycles, each corresponding to 25% of sample strain, were performed at a constant crosshead speed of 1 mm s^{-1}, using a probe 60 mm in diameter. Force-time curves were recorded by the Exponent software to facilitate data capture, storage, and real-time analysis. The parameter hardness (N) refers to the peak force during the first bite. The texture measurement was carried out at 45 °C in order to correspond to the consumption temperature and to avoid bias linked to changes of state during the cooling of the sample. For measurement, three tubers per genotype were utilized. Only the central part of the tuber was selected for analysis in order to maintain consistency and standardization throughout the experiment. From the central part of each tuber, three 23 mm cubes were cut and steam-cooked in a pressure cooking (Moulinex Inicio xl, 3 L, 1000 W; https://www.moulinex.fr/p/inicio-2-15c/8000035882) for 15 min. Before measuring, the cooked cubes were cooled for 7 min to reach 45 °C. Hardness is expressed in N and defined as the strength needed for the cube to be compressed and deformed. TPA measurements were made on tubers from Godet only. Due to limited resources, tubers from Roujol could not be processed on time for a valid comparative analysis.

2.6. Genome wide association studies and characterization of alleles

Genotyping data from a panel of 52 diverse genotypes was obtained from our previous study (Dossa et al., 2023). A genome-wide association study (GWAS) was conducted using 1.9 M high-quality single nucleotide polymorphisms (SNPs), and 45,000 randomly selected SNPs from the initial 1.9 M datasets. The two SNP densities were tested to highlight the effect of SNP density on GWAS power. The SNP density plot was generated using the "CMplot" package (Yin, 2020) in R4.0.23. GWAS was performed using two statistical models: Fixed and random model circulating probability unification (FarmCPU) and mix linear model. The FarmCPU algorithm has enhanced statistical power and computational efficiency and can mitigate false associations in GWAS (Dossa et al., 2023). To identify SNPs significantly associated with traits in each environment, the adjusted p-value was utilized. A significance threshold of $P < 10^{-8}$ (0.05/n, with n being the number of SNPs) was employed to identify significant associations. Additionally, quantile-quantile (QQ) plots were constructed to assess the extent to which the models accounted for population structure by comparing the observed p-values to the expected p-values under the null hypothesis of no association.

2.7. Statistical analysis

The impact of alleles at significant SNPs was evaluated by comparing phenotyping data among different genotypic groups. Student's t-test, with a significance level of $p < 0.05$, was conducted using XLStat 2023.5.1.1399 to compare the genotypic groups.

To analyze genotype by environment interactions, a two-way analysis of variance (ANOVA) was performed using the statistical software Statistical Analysis System (SAS 9.4, SAS Institute Inc.), with genotype and environment as the main effects. A significant genotype × environment interaction indicated differential phenotypic response across environments. Broad sense heritability was estimated by obtaining variance components through Restricted maximum likelihood in ASReml and taking the ratio of genetic variance to the total phenotypic variance. Pearson's correlation coefficients between trait pairs were calculated using standard functions in SAS 9.4, SAS Institute Inc. Together, these analyses enable the characterization of genotype-by-environment effects, total genetic contribution to traits, and trait correlations to elucidate the genomic factors underlying complex phenotypic patterns.

Comparative *in silico* analysis of quantitative trait loci for apparent dry matter content in *D. alata*.

To construct a physical map of quantitative trait loci (QTL) for DM in *D. alata*, marker sequences were obtained from two previous QTL mapping studies by Bredeson et al. (Bredeson et al., 2022) and Gatarira et al. (Gatarira et al., 2020). The marker sequences were then aligned to the *D. alata* V2 reference genome assembly (Bredeson et al., 2022) using BLAST to determine the specific physical positions of the reported QTL regions. Additionally, quantitative trait nucleotides (QTNs) identified from GWAS in the current study were mapped to precise genome locations. All collected QTL positions and QTNs associated with DM were integrated into a comprehensive physical map using Mapchart version 2.32. This consolidated physical map provides a genomic framework for further characterization of sequence variants and candidate genes influencing DM in *D. alata*.

2.8. Expression profiling of candidate genes

Transcriptomic data from tubers of three contrasting genotypes, including Roujol49, Roujol75, and Roujol9, with three biological replicates (Mota et al., 2024), were utilized to characterize the putative candidate genes based on their corresponding expression profiles. The genotypes were selected, based on their culinary qualities and corresponding differential alleles at the peak SNPs, to further investigate the comparative transcriptomic profile and identify putative candidate genes related to each trait under study. A gene was considered differentially expressed if there was a statistically significant difference with a threshold of $\log_2$ fold change $|\log_2 FC| \geq 1$, false discovery rate adjusted p-value (padj) ≤ 0.05 in pairwise comparisons.

3. Results

3.1. Phenotypic characterization of the diversity panel

Table S1 presents data on 52 genotypes of yams and their corresponding measurements for traits such as tuber apparent dry matter content (DM), cooking time, moldability of pounded yam, and hardness of boiled yam (Fig. 2 and Table S1). The mean values for these traits were 29.12% for DM, 18.50 min for cooking time, 5.30 for moldability, and 28.22 N for hardness. The range of values varied across the genotypes, with the minimum and maximum values being 20.55% and 37.28% for DM, 11 min and 33 min for cooking time, 1.67 and 9 for moldability, and 6.39 and 85.73 N for hardness, respectively. The coefficient of variation (CV) indicated the degree of variation within the traits, with CV values ranging from 10.54 to 60.50%. These results provide valuable insights into the diversity of *D. alata* genotypes in relation to their cooking quality, which can be harnessed in breeding programs.

Overall, the observed differences in traits between Roujol and Godet indicate the environmental effects on the culinary characteristics of *D. alata* (Table 1). These differences could be attributed to variations in environmental conditions, soil composition, or other factors specific to each location.

We further estimated the correlation coefficients between the traits (Fig. 2H). The correlation analysis for *D. alata* traits reveals that DM is strongly and positively correlated with moldability and hardness. This observation means *D. alata* varieties with higher apparent dry matter content exhibit better moldability and higher hardness. Cooking time shows a weaker correlation with moldability and hardness. Moldability and hardness in yam are strongly and positively correlated, indicating that boiled yam hardness can be used to predict moldability quality. Moreover, cooking time and moldability showed high heritabilities, 0.89 and 0.93, respectively, implying that breeding for these traits in *D. alata* will be amenable.

3.2. Impact of SNP density and statistical model on GWAS result

In our previous study, we performed a comprehensive genetic analysis on our diversity panel consisting of multiple genotypes and showed a low structure and its suitability for GWAS (Dossa et al., 2023). In order to determine how statistical models and SNP density affect GWAS, we selected two traits, cooking time and DM, and performed GWAS with two SNP densities: low density with 45,000 SNPs (Fig. 3A, 0.09 SNPs/kb) and high density with 1.9 M SNPs (3.9 SNPs/kb)) as well as two statistical models (FarmCPU and MLM). The results showed that high-density SNP data improves GWAS power with highly significant associations ($-\log_{10}P > 9$), as depicted in Fig. 3B and C. Additionally, the FarmCPU model outperformed the MLM model with more significant associations ($-\log_{10}P > 7.5$), as shown in Fig. 3D and E. These findings suggest that utilizing high-density SNP data and employing the FarmCPU model enhances the accuracy and power of GWAS in *D. alata*. The 1.9 M SNPs dataset was thus kept for downstream analyses.

3.3. GWAS for apparent dry matter content

GWAS for tuber DM identified six significant associations ($Log_{10}P > 9$) at the two locations, Roujol and Godet (Table 2). In Roujol, significant associations were found on chromosomes 1, 3, 9, 17, and 20 (Fig. 4A and B), while in Godet, a significant association was observed on chromosome 9 (Fig. 4C and D). Interestingly, the genomic region on

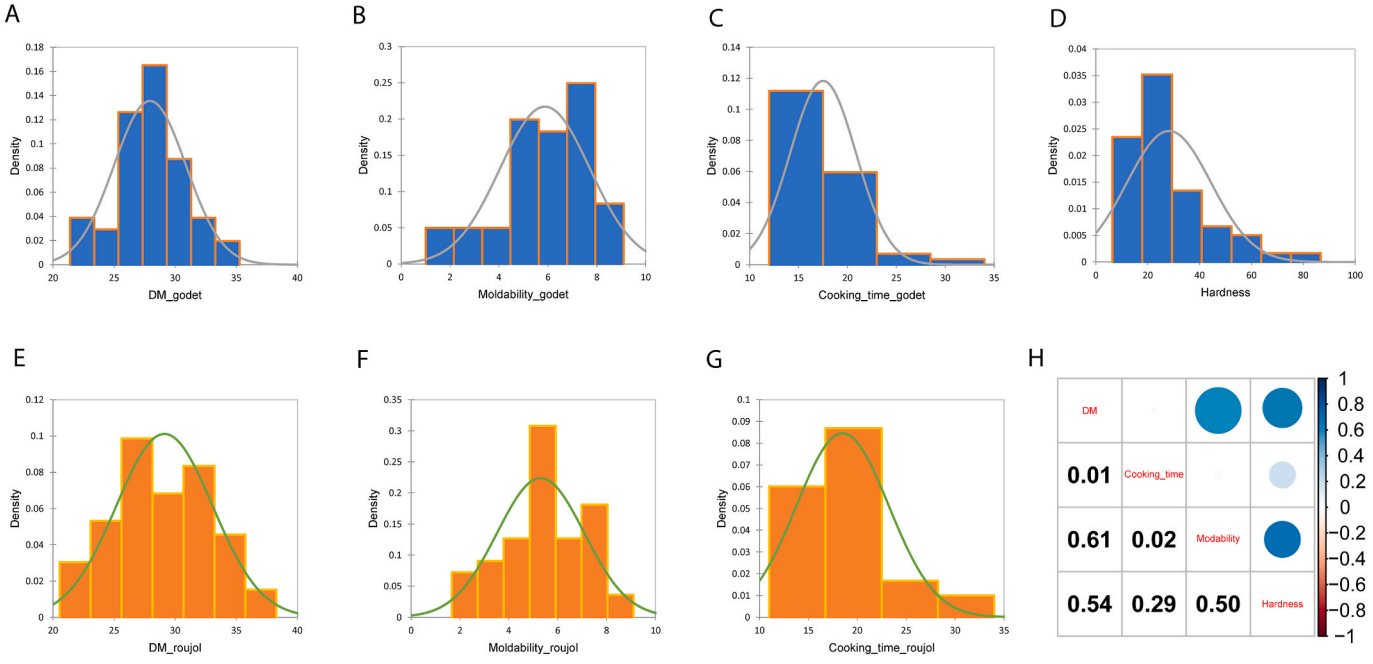

Fig. 2. Phenotypic variation estimated for traits *D. alata* culinary traits. A) Apparent dry matter content (DM) at Godet, **B)** Moldability at Godet, **C)** Cooking time at Godet, **D)** Hardness at Godet, **E)** DM at Roujol, **F)** Moldability at Roujol, **G)** Cooking time at Roujol, and **H)** Correlation between the different traits under study.

Table 1
Statistics of the phenotypic traits among the varieties and planting sites.

Traits	Mean		Standard deviation		Minimum		Maximum		CV		G	E	G × E	H^2
Sites	R	G	R	G	R	G	R	G	R	G				
Cooking time (min)	18.5	17.52	4.72	3.37	11	12	33	33	25.5	19.25	***	***	***	0.89
DM (%)	29.12	27.94	3.94	2.94	20.55	21.39	37.28	34.26	13.54	10.53	***	**	**	0.69
Hardness (N)	28.22		17.07		6.39		85.73		60.5		***	–	–	–
Moldability	5.3	5.88	1.78	1.84	1.67	1	9	9	33.62	31.28	***	***	***	0.93

Note. Where R stands for Roujol and G stands for Godet. While "***" indicate significance at $p < 0.001$ and "**" indicate significance at $p < 0.01$.

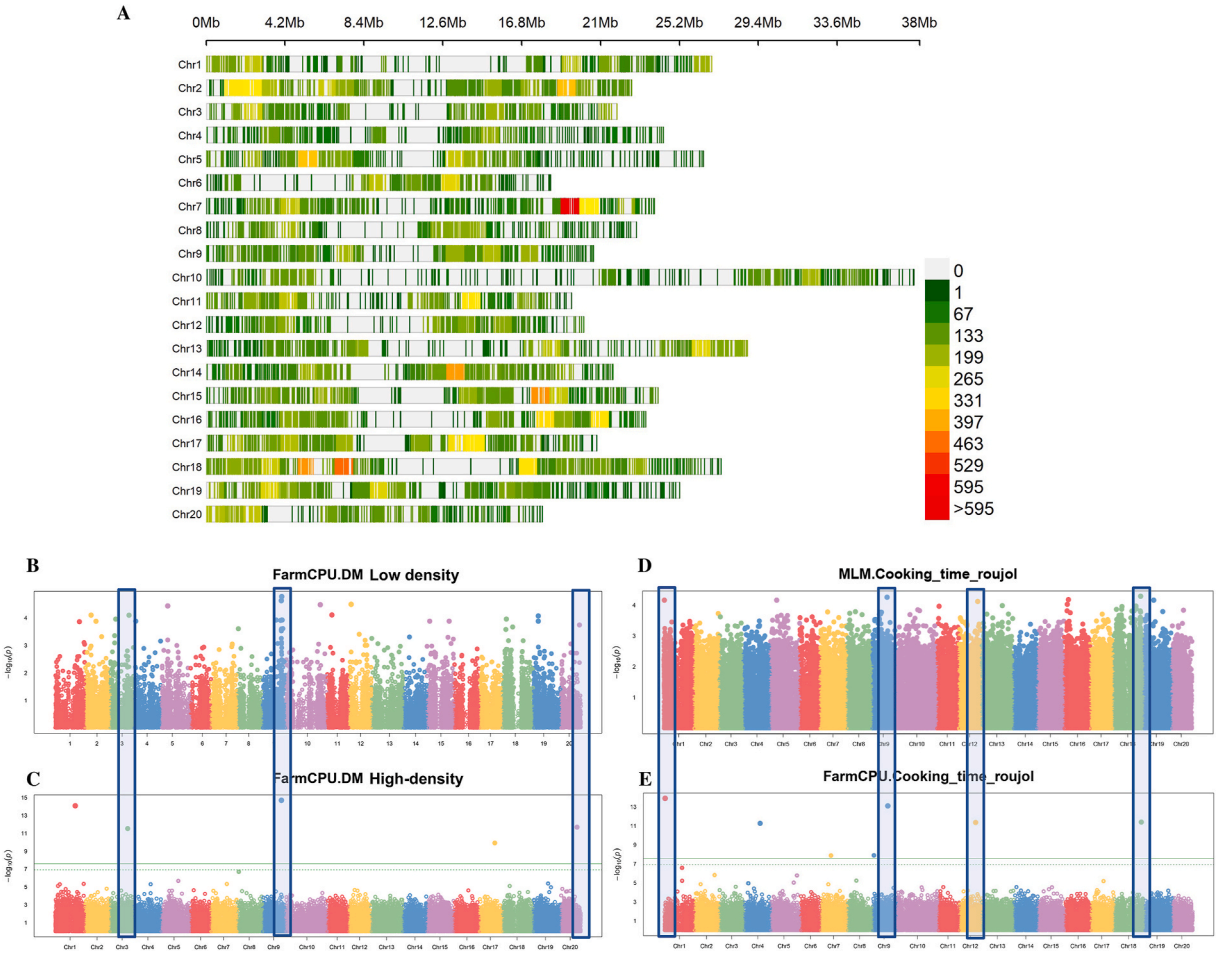

Fig. 3. Effect of SNP density and model selection on GWAS. A) subplot A displays the SNP density at a lower level, with 45,000 SNPs. The plot with 1.9 M SNPs was presented by Dossa et al. (Dossa et al., 2023). This plot illustrates the distribution and density of SNPs across the genome, indicating the coverage and resolution of the genotyping data used in the GWAS analysis **B)** The Manhattan plot in subplot B represents the GWAS associations obtained using the lower SNP density. The Manhattan plot shows the genomic positions of SNPs on the x-axis, with their corresponding association p-values on the y-axis. The peaks in the plot indicate significant associations between SNPs and the studied trait. The lower SNP density may result in fewer significant associations being detected**, C)** Manhattan plot showing GWAS associations with high SNP density. This plot represents the GWAS using a higher density of SNPs, which improves the coverage and resolution of the genotyping data. With higher SNP density, more potential associations between SNPs and the trait can be identified, **D)** Manhattan plot showing GWAS associations estimated using mixed linear model (MLM) for cooking time at Roujol, **E)** Manhattan plot showing GWAS associations estimated using FarmCPU for cooking time at Roujol.

chromosome 9 was consistently identified in both locations. The variation in GWAS results between the two locations could be attributed to environmental factors impacting the phenotype. To gain further insights into the effects of the identified loci, allelic effects for the top significant or stable SNPs (rs839254 and rs50615) were investigated using allele segregation analysis.

The SNP rs839254 consistently appeared in the same genomic region on chromosome 9 at both locations (Table 2). It exhibited clear differentiation of genotypes into three allelic groups: AA, GA, and GG. Homozygous accessions (AA) at this locus were associated with higher DM compared to AG and GG genotypes (Fig. 4F). SNP rs839254 is annotated as a downstream SNP of *Dioal.09G047900*, which encodes a Winged helix-turn-helix DNA-binding domain protein (Table 3).

Additionally, we characterized another significant SNP, rs50615 ($Log_{10}P = 14.08$), specifically identified at the Godet location (Fig. 4A). Notably, both genotypes GA and GG exhibited significant differences in DM (Fig. 4E), with the GA genotype associated with higher DM. The SNP rs50615 is annotated as an intergenic SNP located between the genes *Dioal.01G035200* (No apical meristem-associated *C*-terminal domain (NAM-associated)) and *Dioal.01G035300* (Zinc-binding dehydrogenase

Table 2
The significant signals identified with the allelic effects for each SNP.

No	Traits	Site	SNP	CHR	Position	Allele	-log$_{10}$P	Effect
1	Apparent dry matter content	Godet	rs839254	Chr9	16,943,384	**AA**/GA/GG	14.7	−2.16
2		Godet	rs50615	Chr1	18,210,046	**GA**/GG	14.08	3.34
3		Godet	rs1949352	Chr20	14,439,520	A/G	11.69	−2.29
4		Godet	rs287772	Chr3	15,958,362	C/T	11.53	−2.54
5		Godet	rs1627402	Chr17	13,837,380	C/A	9.92	−1.4
6		Roujol	rs839254	Chr9	16,943,384	**AA**/GA/GG	13.2	−2.05
7	Hardness	Godet	rs1634685	Chr17	15,518,325	**TC**/TT	29.97	−26.22
8		Godet	rs1025607	Chr11	8,468,326	GA/**GG**	13.53	−5.43
9		Godet	rs1712814	Chr18	15,399,183	T/C	13.1	−7.54
10		Godet	rs1766280	Chr19	900,300	G/A	12.87	−6.95
11		Godet	rs1038631	Chr11	10,920,503	C/T	9.67	5.05
12		Godet	rs1151977	Chr13	611,665	C/T	8.51	5.33
13	Moldability	Godet	rs381001	Chr4	18,391,273	AA/**CC**/AC	11.55	4.74
14	Cooking time	Godet	rs2792	Chr1	583,131	GA/**GG**	28.92	12.14
15		Godet	rs1242616	Chr13	20,161,008	GG/GT/**TT**	13.78	1.86
16		Godet	rs1358344	Chr15	1,392,567	T/C	8.22	−1.08
17		Godet	rs1743358	Chr18	22,503,030	C/T	8.11	1.28
18		Godet	rs196262	Chr2	21,385,799	A/C	10.99	−1.58
19		Roujol	rs2792	Chr1	583,131	GA/**GG**	13.9	6.67
20		Roujol	rs821713	Chr9	13,328,572	AA/**TA**	13.1	3.48
21		Roujol	rs1751594	Chr18	24,585,447	AA/AT/TT	11.39	2.74
22		Roujol	rs1128334	Chr12	14,577,742	TG/TT	11.35	−2.02
23		Roujol	rs363258	Chr4	14,556,249	GA/GG	11.27	2.25
24		Roujol	rs754523	Chr9	815,494	GG/GT	7.89	1.57
25		Roujol	rs615299	Chr7	9,054,657	CC/CT	7.87	1.86

Alleles in bold have be found to contribute positively to the trait.

family protein) (Table 3).

3.4. GWAS for cooking time

The GWAS for cooking time revealed eleven significant associations ($Log_{10}P > 7$) at the Roujol and Godet locations (Table 2). In Roujol, significant associations were observed on chromosomes 1, 3, 13, 15, and 14 (Fig. 5A and B), while in Godet, significant associations were found on chromosomes 1, 4, 7, 9, 12, and 18 (Fig. 5C and D). One genomic region on chromosome 1 showed consistent associations in both locations. To explore the effects of these identified loci, we performed an allele segregation analysis on the top significant or stable SNP, rs2792.

The significant SNP rs2792 displayed distinct genotypic differences, with two alleles identified: GA and GG. Accessions with heterozygous genotypes (GA allele) at this locus exhibited longer cooking time compared to those with GG allele (Fig. 5E). The SNP rs2792 is annotated as an intergenic SNP located between the genes *Dioal.01G006400* (Tripeptidyl peptidase) and *Dioal.01G006500* (Proprotein convertase subtilisin) (Table 3).

3.5. GWAS for hardness and moldability

For hardness, several significant associations were identified. The SNPs rs1634685 on Chr17, rs1025607 on Chr11, rs1712814 on Chr18, rs1766280 on Chr19, rs1038631 on Chr11, and rs1151977 on Chr13 exhibited strong associations with hardness (Fig. S1C). These SNPs had high effect sizes, indicating their influence in determining the hardness of the boiled yam. Allele segregation analysis further confirmed the differentiation of genotypes into two groups based on the TC and TT alleles at SNP rs1634685 (Fig. S2A). Moreover, several candidate genes associated with these SNPs were identified as *Dioal.17G070100, Dioal.17G070200, Dioal.11G03900, Dioal.18G037600, Dioal.18G037700, Dioal.19G012800, Dioal.11G040300, Dioal.11G040400, Dioal.13G006900,* and *Dioal.13G007000* (Table 3).

In the case of moldability, the SNP rs381001 on Chr4 showed a significant association (Figs. S1A and S1B). This SNP had three different alleles (AA, CC, AC), and individuals with the AC allele had medium moldability scores (Fig. S2B). The effect size for this SNP was positive, indicating that the AC allele contributes to increased moldability. Furthermore, rs381001 was identified as a non-synonymous SNP in the exonic region of *Dioal.04G099800*, annotated as a Steroid-transporting ATPase (Table 3). Further investigations are needed to determine the precise role of this gene in relation to moldability.

3.6. In silico comparative analysis of genomic regions identified from previous studies on apparent dry matter

To gain a comprehensive understanding of the genetic control of the DM trait, we compared the identified quantitative trait loci (QTL) from our study with those reported in two previous studies (Bredeson et al., 2022; Gatarira et al., 2020). Surprisingly, we found no overlap between the QTLs identified in the three studies (Fig. S3). Although some of the parental genotypes used in the study by Bredeson et al. (Bredeson et al., 2022) were also present in the study by Gatarira et al. (Gatarira et al., 2020), none of the QTLs were found to coincide. This finding further emphasizes the complex nature of DM, which appears to be influenced by multiple genomic regions and potentially affected by interactions between genotypes and the environment.

3.7. Expression profiling of candidate genes

Transcriptome data (Mota et al., 2024) of multiple allele-specific genotypes with contrasting phenotypes were analyzed to explore the expression profile of the putative genes detected around the peak SNPs. Out of the 42 candidate genes identified near the peak SNPs on different chromosomes, a subset of 3 and 2 genes showed differential expression pattern for cooking time and apparent dry matter contents, respectively (Fig. 6A–B). *Dioal.02G085400* (rs196262), *Dioal.04G067700* (rs363258), and *Dioal.09G012500* (rs754523), identified as candidate genes for cooking time, showed differential expression (Upregulated in Roujol49 with higher cooking time (32 min) compared to Roujol9 with lower cooking time (15.33 min)). *Dioal.02G085400*, *Dioal.04G067700*, and *Dioal.09G012500* encode Threonine protein kinase, ABL interactor-like protein 2, and Transposon protein, respectively (Table 3). It can be speculated from the results that higher expression of these putative candidate genes result in physiological changes leading to longer cooking time.

Moreover, concerning apparent dry matter content, two candidate

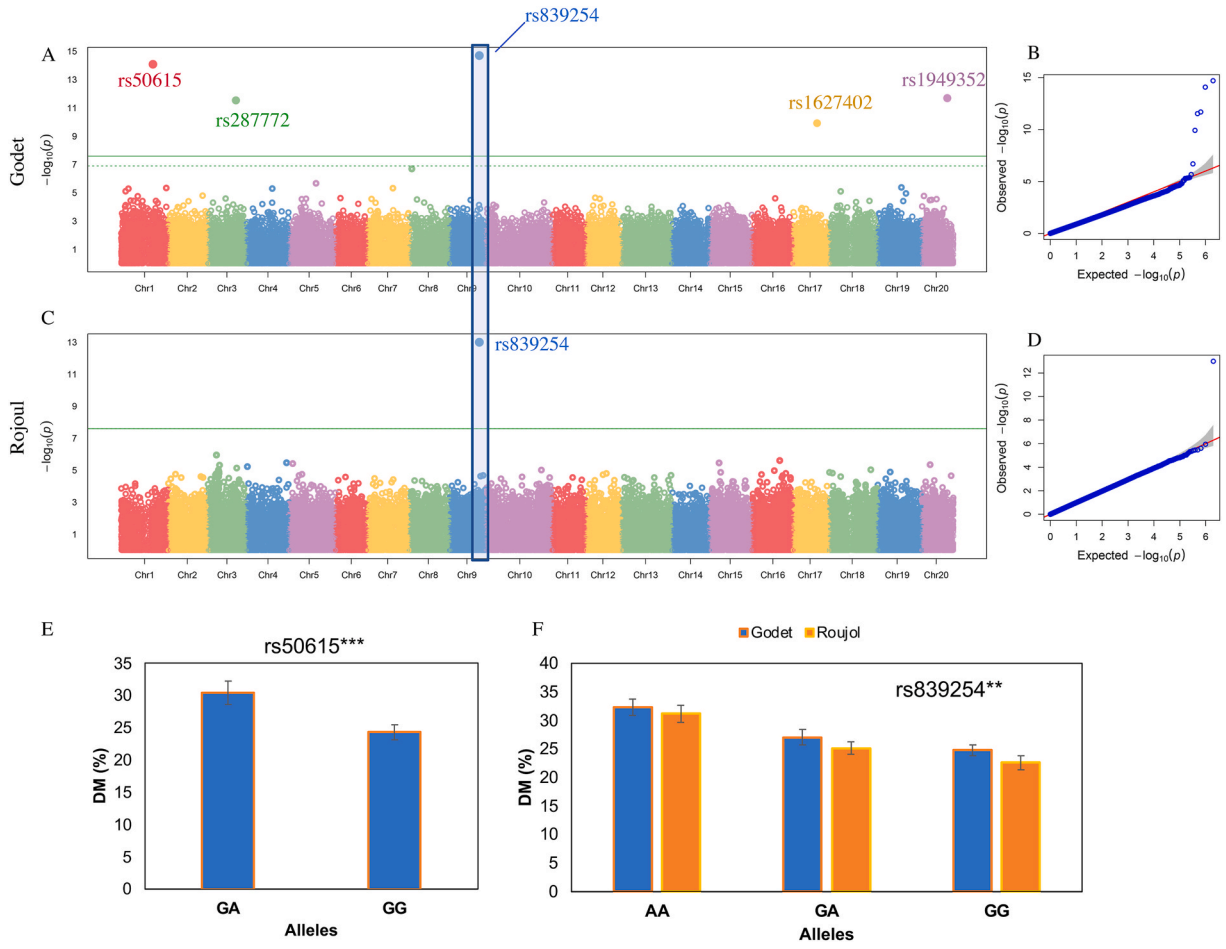

Fig. 4. GWAS for apparent dry matter content (DM). A) The Manhattan plot for DM at Godet shows the genomic positions of SNPs plotted against their association p-values. The peaks in the plot indicate significant GWAS signals. The green horizontal lines represent the genome-wide significance threshold, helping to identify SNPs that surpass the threshold and are considered highly associated with DM, **B)** The QQ-plot associated with DM at Godet depicts the observed p-values against the expected p-values under the null hypothesis of no association. Deviations from the diagonal line suggest potential associations between SNPs and DM, **C)** The Manhattan plot for DM at Roujol displays the genomic positions of SNPs and their association p-values specifically for the DM at Roujol, **D)** The QQ-plot associated with DM at Roujol is similar to the plot described in (B), **E)** The allele segregation analysis concerning SNP rs50615 demonstrates the relationship between different genotypes and their corresponding cooking times. In this analysis, the GA allele is associated with genotypes that have higher cooking time, while the GG allele is associated with genotypes that have lower DM, **F)** The allele segregation analysis concerning SNP rs839254 demonstrates the relationship between different genotypes and their corresponding cooking times. In this analysis, the GA allele is associated with genotypes that have medium DM, and the AA allele is associated with genotypes that have higher DM, while the GG allele is associated with genotypes that have lower DM.
** indicates significant differences at p = 0.01 and *** corresponds to significant difference at p = 0.0001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

genes, *Dioal.01G035200* and *Dioal.20G055500*, were identified with differential expression patterns in contrasting genotypes (Roujol75 vs. Roujol9). Roujol75 has a lower DM (26.67 %) compared to Roujol9 (33.77 %).

4. Discussion

The culinary quality of yam is a crucial aspect that determines consumers' choices and various processing purposes (Barlagne et al., 2017). Understanding and improving yam quality is of great importance to farmers, consumers, and the yam industry (Honfozo et al., 2021; Otegbayo et al., 2023). It involves the identification of genetic factors, environmental influences, and processing techniques that can enhance desirable quality traits and ensure consistent product performance. Through genetic studies, sensory evaluations, and processing optimization, efforts are being made to enhance yam quality and promote its utilization as a nutritious staple worldwide (Bredeson et al., 2022; Dossa et al., 2023; Gatarira et al., 2020; Honfozo et al., 2021; Mota et al., 2024; Otegbayo et al., 2023).

In this study, the observed phenotypic variation between Roujol and Godet in terms of quality traits in *D. alata* can be attributed to environmental influences. Previous studies showed that environmental factors significantly influence yam yield (Asfaw, 2016; Norman et al., 2022), but multi-location trials assessing environmental impacts on quality traits in *D. alata* are lacking (Dossa et al., 2023). Conducting multi-location trials across diverse environments is crucial to fully understand genotype-by-environment interactions and identify stable, high-quality genotypes (Crossa, 1990). Moreover, establishing trait correlations can reveal proxy to improve the prediction of yam quality. For instance, correlating quality traits like apparent dry matter content, starch composition, and texture profiles with sensory and cooking properties could reveal proxy traits that predict eating quality (Ehounou et al., 2021; Honfozo et al., 2021; Li et al., 2024; Otegbayo et al., 2023). This offers a path to accelerated breeding gains for culinary quality without exhaustive cooking assays. A previous work by Ehounou et al. (Ehounou et al., 2021) has proposed that high protein content, low hardness, and high cohesiveness may contribute to reduced moldability in yam tubers. Based on these findings, they suggested using these

Table 3
Summary of identified candidate genes associated with tuber quality traits in *Dioscorea alata*.

No	Traits	SNP	Position	Upstream/Downstream gene	Localization	Predicted function	Planting site[a]
1	Moldability	rs381001	18,391,273	*Dioal.04G099800*	exonic (synonymous)	Steroid-transporting ATPase	G
2	Cooking time	rs2792	583,131	*Dioal.01G006400*	intergenic	Tripeptidyl peptidase	G
3				*Dioal.01G006500*		Proprotein convertase subtilisin	G
4		rs1242616	20,161,008	*Dioal.13G064800*	intergenic	Myb_dna-bind_3	G
5				*Dioal.13G064900*		Cucumisin	G
6		rs1358344	1,392,567	*Dioal.15G018400*	intronic	Threonine protein kinase	G
7		rs1743358	22,503,030	*Dioal.18G053100*	intergenic	Rna-dependent RNA polymerase 1	G
8				*Dioal.18G053200*		Rna-dependent RNA polymerase 1	G
9		rs196262	21,385,799	*Dioal.02G085300*	intergenic	Threonine protein kinase	G
10				*Dioal.02G085400*		Threonine protein kinase	G
11		rs2792	583,131	*Dioal.01G006400*	intergenic	Tripeptidyl peptidase	R
12						Proprotein convertase subtilisin	R
13				*Dioal.01G006500*			
14		rs821713	13,328,572	*Dioal.09G034300*	intronic	PPR repeat domain containing protein	R
15		rs1751594	24,585,447	*Dioal.18G073800*	downstream	Calcium-responsive transcription coactivator	R
16		rs1128334	14,577,742	*Dioal.12G041900*	intergenic	Plant protein of unknown function domain containing protein	R
17				*Dioal.12G042000*		Sec14 protein-like	R
18		rs363258	14,556,249	*Dioal.04G067700*	intronic	ABL interactor-like protein 2	R
19		rs754523	815,494	*Dioal.09G012400*	intergenic	CYTOCHROME P450 (phenylalanine *n*-monooxygenase (CYP79A2))	R
20				*Dioal.09G012500*		Transposon protein	R
21		rs615299	9,054,657	*Dioal.07G056700*	intergenic	Uncharacterized UPF0114 domain containing protein	R
22				*Dioal.07G056800*		Glutathione s-transferase THETA 1	R
23	Hardness	rs1634685	15,518,325	*Dioal.17G070100*	intergenic	Regulatory component of ABA receptor 1	G
24				*Dioal.17G070200*			
25						Translation elongation factor EF1B/ribosomal protein S6 family protein	G
26		rs1025607	8,468,326	*Dioal.11G039000*	upstream	Ribosomal protein s7p/s5e family protein	G
27		rs1712814	15,399,183	*Dioal.18G037600*	intergenic	Na	G
28				*Dioal.18G037700*		*S*-adenosyl-l-methionine-dependent methyltransferase	G
29		rs1766280	900,300	*Dioal.19G012800*	upstream	Leucine-rich repeat protein	G
30		rs1038631	10,920,503	*Dioal.11G040300*	intergenic	Phosphoenolpyruvate carboxylase-related kinase 1	G
31				*Dioal.11G040400*		Na	G
32		rs1151977	611,665	*Dioal.13G006900*	intergenic	Threonine protein kinase	G
33				*Dioal.13G007000*		Threonine protein kinase	G
34	Apparent dry matter content	rs839254	16,943,384	*Dioal.09G047900*	downstream	Winged helix-turn-helix DNA-binding domain protein	G
35		rs50615	18,210,046	*Dioal.01G035200*	intergenic	No apical meristem-associated *C*-terminal domain (NAM-associated)	G
36				*Dioal.01G035300*			
37						Zinc-binding dehydrogenase family protein	G
38		rs1949352	14,439,520	*Dioal.20G055500*	Exonic (non-synonymous)	Protein kinase domain	G
39		rs287772	15,958,362	*Dioal.03G055700*	upstream	Heat shock protein dnaj	G
40		rs1627402	13,837,380	*Dioal.16G053800*	intergenic	Na	G
41				*Dioal.16G053900*		Glutamine dumper 4	G
42		rs839254	16,943,384	*Dioal.09G047900*	downstream	Winged helix-turn-helix DNA-binding domain protein	R

[a] G represents Godet while R represents Roujol.

criteria for selecting moldable genotypes in breeding programs. Our study reveals that DM is associated with improved moldability and increased hardness in yam tubers. Strong correlation and high heritability suggest DM as a robust proxy for accelerating indirect improvement of yam culinary quality, complemented by other potential proxies like starch and texture. Moreover, our diversity panel exhibits sufficient variation for multiple quality traits, enabling association mapping.

The presence of a high degree of recombination in *D. alata*, as suggested in a previous report (Dossa et al., 2023), underscores the importance of utilizing high SNP density for GWAS in yam. We validated this by conducting GWAS using low-density (45,000 SNPs) and high-density (1.9 million SNPs) SNP datasets. Our findings confirmed that high SNP density significantly improves the GWAS results in *D. alata*. This high resolution allowed for more precise identification of associations between genetic markers and the studied traits. In addition, our results also demonstrated that the multi-locus FarmCPU model outperformed the single-locus MLM model in terms of identifying key associations between genetic markers and the studied traits. These findings are consistent with previous reports highlighting the superior performance of multi-locus GWAS models compared to single-locus models (Liu, Huang, Fan, Buckler, & Zhang, 2016; Zhang, Jia, & Dunwell, 2019).

Food texture plays a crucial role as a quality indicator in various food products, including boiled and pounded yam (Otegbayo et al., 2021). It encompasses the structural and mechanical properties of the food, as well as the sensory perception experienced during handling and consumption. Traits such as DM, fiber percentage, mealiness, waxiness, stickiness, and hardness are important textural attributes for boiled yam (Otegbayo, Oroniran, Fawehinmi, & Ayandiji, 2019), which vary across different genotypes and environments (Ezeocha, Nwankwo, & Ezebuiro, 2015; Otegbayo et al., 2019). Bredeson et al. (Bredeson et al., 2022) identified a QTL on chromosome 18 associated with DM in *D. alata*.

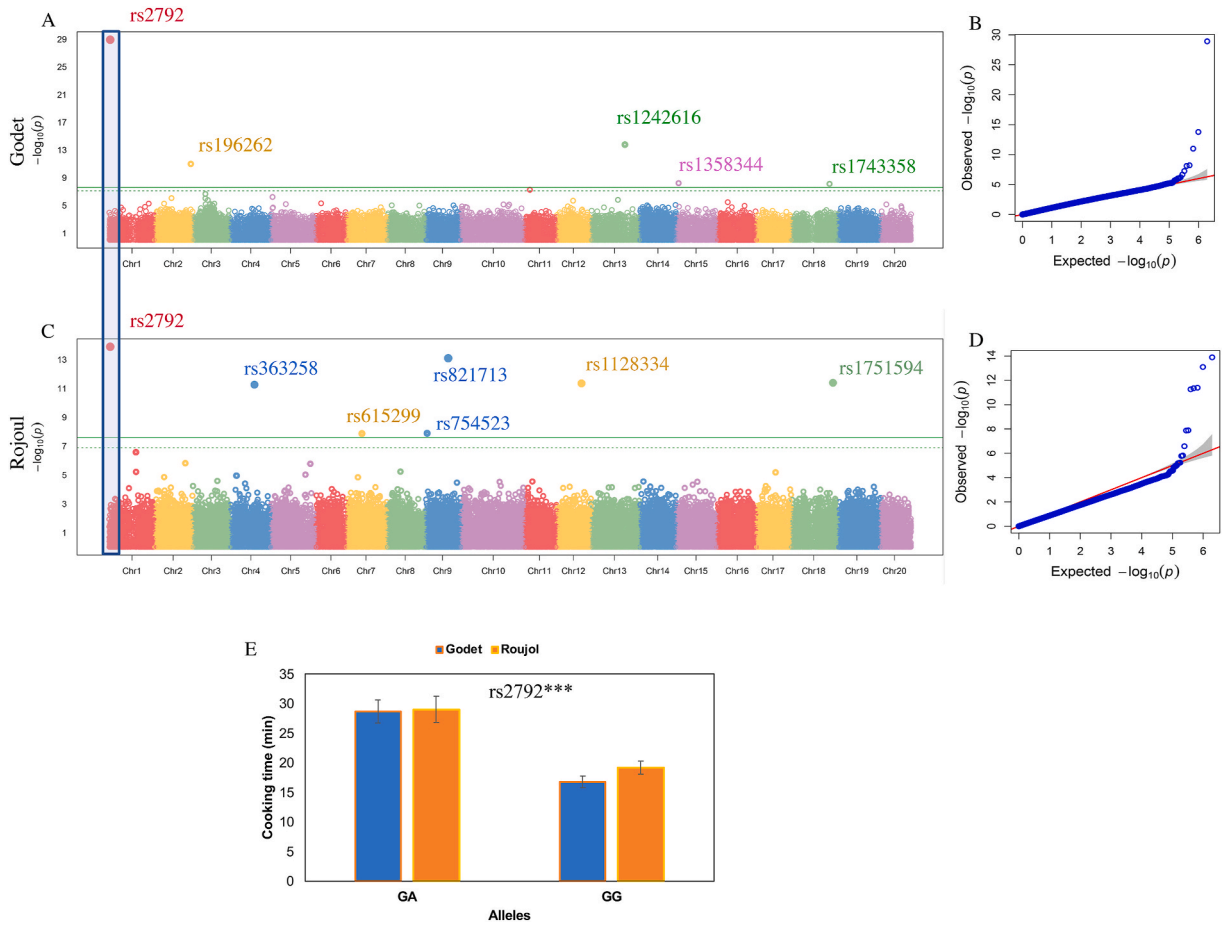

Fig. 5. GWAS for cooking time in *D. alata*. A) The Manhattan plot for the cooking time at Godet shows the genomic positions of SNPs plotted against their association p-values. The peaks in the plot indicate significant GWAS signals. The green horizontal lines represent the genome-wide significance threshold, helping to identify SNPs that surpass the threshold and are considered highly associated with cooking time, **B)** The QQ-plot associated with cooking time at Godet depicts the observed p-values against the expected p-values under the null hypothesis of no association. Deviations from the diagonal line suggest potential associations between SNPs and cooking time, **C)** The Manhattan plot for cooking time at Roujol displays the genomic positions of SNPs and their association p-values specifically for the cooking time at Roujol, **D)** The QQ-plot associated with cooking time at Roujol is similar to the plot described in (B), **E)** The allele segregation analysis concerning SNP rs176324 demonstrates the relationship between different genotypes and their corresponding cooking times. In this analysis, the GA allele is associated with genotypes that have long cooking times, while the GG allele is associated with genotypes that have short cooking times.
*** indicate t significant difference at p = 0.0001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

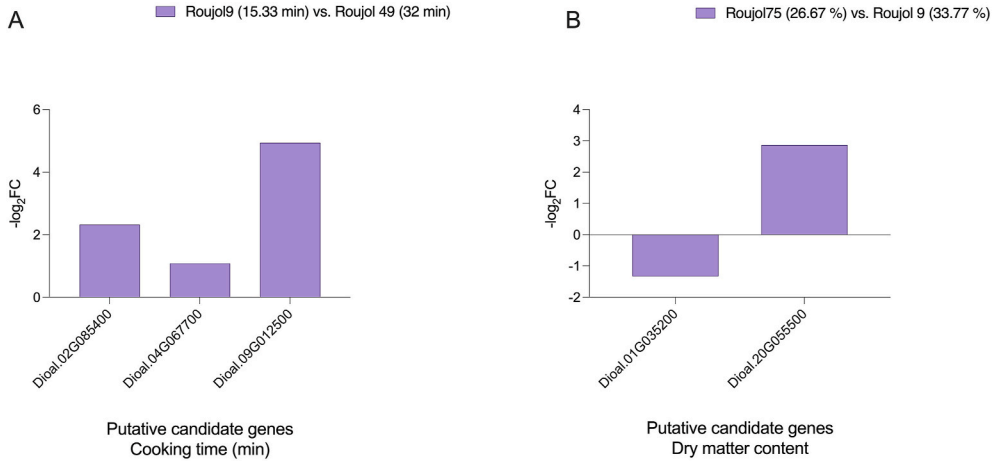

Fig. 6. Differential expression of candidate genes identified in various comparisons of *D. alata* genotypes with contrasting culinary qualities. A) Expression profile of differentially expressed putative candidate genes for cooking time (min) in comparison Roujol9 vs. Roujol49 **B)** Differentially expressed putative candidate genes for apparent dry matter contents in comparison Roujol75 vs. Roujol9. The comparisons were selected based on values in parenthesis for each trait.

Gatarira et al. (Gatarira et al., 2020) studied DM in yam tubers at four locations but did not report stable SNP. In our study, we identified a stable SNP associated with DM on chromosome 9 (SNP rs839254). This SNP is located downstream of the *Dioal.09G047900* gene, which encodes a Winged helix-turn-helix DNA-binding domain protein. We propose that *Dioal.09G047900* may play a significant role for DM content in *D. alata*. Two differentially expressed genes identified between high and low apparent dry matter genotypes encode a No Apical Meristem-associated *C*-terminal domain (NAM) transcription factor (*Dioal.01G035200*; SNP rs50615) and a protein kinase domain-containing protein (*Dioal.20G055500*; SNP rs1949352). NAC transcription factors regulate diverse growth, developmental, and stress response processes in plants (Zhang et al., 2018), while protein kinases play critical roles in signaling cascades and cellular regulation (Chen et al., 2021). For instance, a previous report found that overexpression of a calcium-dependent protein kinase in potato supported tuber wound healing (Ma et al., 2022). However, neither of these genes has been functionally characterized in yam. Further investigation and molecular studies are warranted to elucidate the potential mechanisms by which these differentially expressed genes may modulate DM in *D. alata*. There are limited tools available currently to test candidate genes, and GWAS hits in yams (Syombua et al., 2021). For instance, CRISPR-Cas genome editing system could be very useful for improving yams through functional genomics (Feng et al., 2018; Zaman et al., 2023). "Cut-dip-budding" delivery system (Cao et al., 2023) provides a way to deliver CRISPR-Cas to edit and study these genes without the need for transformation and regeneration and enables rapid, efficient CRISPR editing while minimizing off-target effects and eliminating transgenes.

Similarly, we identified a stable SNP for cooking time on chromosome 1. This particular SNP was located in an intergenic region between two genes, *Dioal.01G006400* and *Dioal.01G006500*. Of these two genes, *Dioal.01G006400* encodes a Tripeptidyl peptidase (TPP) known enzyme that plays an important role in protein breakdown and metabolism in plants (Arima, Uchikoba, Yonezawa, Shimada, & Kaneda, 2000; Silva-López & Gonçalves, 2019). This could have implications for the breakdown of proteins during cooking, potentially affecting the cooking time of yam tubers by manipulating protein turnover (Tomkinson & Lindås, 2005). Moreover, three genes, encoding Threonine protein kinase, ABL interactor-like protein 2, and Transposon protein, identified in the close proximity of GWAS associations were also identified as DEGs in two contrasting genotypes for cooking time.

The identification of *Dioal.04G099800*, which encodes a Steroid-transporting ATPase protein, in the vicinity of the association signal on chromosome 4 suggests its potential role in regulating moldability in *D. alata*. ABC transporters, including their ATP-binding proteins, are known for their involvement in the transport of various molecules across cellular membranes (Yazaki, 2006). For example, in some root crops, the ABC transporters are involved in the transport of starch, which is a major component contributing to the hardness or firmness of the roots (Singh, Selvakumar, Mangal, & Kalia, 2020). These transporters could play a role in the uptake and storage of starch in tubers, influencing the texture, moldability, and firmness of the tubers.

In this study, transcriptomic analysis was performed on mature tubers, which precluded the identification of DEGs during earlier developmental stages that may influence trait variation. Additionally, many genotypes shared the same alleles at peak SNPs identified through GWAS, limiting pairwise comparisons. To better elucidate DEGs and validate candidate genes, future studies could examine gene expression at multiple tuber developmental time-points using RNA-seq and qRT-PCR (Li et al., 2024). Comparing genotypes with contrasting alleles at peak GWAS SNPs would help connect transcriptional profiles to sequence polymorphisms influencing the phenotype. Examining immature tubers would provide greater insight into transcriptional regulation during critical stages of tuber formation and expansion.

The GWAS peaks and putative candidate genes identified in this study represent novel findings in the context of *D. alata* and their association with quality attributes. These findings expand our understanding of the genetic basis of the culinary attributes of *D. alata*. Future studies should expand beyond core traits like DM content to explore additional biochemical factors affecting texture and taste, such as amylose content and pectin levels (https://rtbfoods.cirad.fr/). Transitioning significant marker-trait associations into KASP or SNP markers will facilitate breeding tasks (Cormier et al., 2021). Additionally, adopting high-throughput phenomic tools like NIRS (Lebot & Malapa, 2013; Van Tassel et al., 2022) modeling for tuber quality traits will accelerate selective breeding. Recent studies demonstrate the potential of NIRS analysis on raw tubers or flours for rapid, nondestructive evaluation of breeding populations (Ehounou et al., 2021; Houngbo et al., 2024; Lebot & Malapa, 2013; Otegbayo et al., 2023). Integrating NIRS-enabled phenotyping into selection pipelines could greatly increase genetic gains for culinary quality. By combining expanded genomics research, biotechnology advances like CRISPR editing, and front-end phenotypic screening, transformative improvements in yam culinary quality are within reach.

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CRediT authorship contribution statement

Komivi Dossa: Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Mahugnon Ezékiel Houngbo:** Validation, Methodology, Formal analysis, Data curation. **Mathieu Lechaudel:** Methodology, Formal analysis, Data curation. **Erick Malédon:** Validation, Methodology, Investigation. **Yedomon Ange Bovys Zoclanclounon:** Software, Methodology, Investigation. **Jean-Luc Irep:** Validation, Methodology, Investigation. **Mian Faisal Nazir:** Software, Methodology, Formal analysis, Data curation. **Hâna Chair:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Denis Cornet:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116301.

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