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Identification and analysis of genomic regions influencing leaf morpho-physiological traits related to stress responses in greater yam (*Dioscorea alata* L.)

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Abstract

Background Yams (*Dioscorea* spp.) are significant food security crops especially in West Africa. With the increasing tuber demand and climate change challenges, it is pertinent to strengthen breeding programs for developing high-yielding cultivars with climate resilience. The current study aimed at deciphering the genetic basis of leaf traits related to stress responses in a diverse panel of *Dioscorea alata* genotypes.

Results Phenotypic characterization of 12 traits, including leaf dry matter content, mean leaf area, net photosynthesis, transpiration rate, transpiration use efficiency, stomatal density, stomatal index, preformed node count, leaf thickness, competitor, stress-tolerator, ruderal ecological strategies emphasized significant variations among the genotypes and across two planting locations. Weak correlations were observed among most of traits, suggesting that breeding simultaneously for some of these stress response-related traits would be possible. Heritability was highest for transpiration rate, leaf area and stomatal density, while it was lowest for stress-tolerator, ruderal ecological strategies. Genome-wide association study (GWAS) using high-quality single nucleotide polymorphism (SNPs) identified 24 significant associations on 11 chromosomes, where the association signals were consistent across two locations for traits with high heritability, viz., stomatal density (Chr18) and transpiration rate (Chr3). Further characterization of the significant signals and their related alleles identified advantageous alleles contributing positively to the studied traits. Moreover, 44 putative candidate genes were identified. *Dioal.18G049300* (3 keto acyl-coenzyme A synthase) was identified as a strong candidate gene for stomatal density, while *Dioal.12G033600* (Phosphatidylinositol monophosphate 5 kinase 4) was identified for net photosynthesis.

Conclusion Taken together, GWAS and allele segregation analysis for key SNPs provided significant insights into the marker-trait associations, which can be further utilized in breeding programs to improve climate resilience in greater yam.

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Keywords Yam, Marker-trait association, Physiology, Breeding, Climate resilience

Background

Crop improvement involves population development, identification of key traits, phenotyping, deciphering the genetics, and combining the traits of interest [1–3]. Although classical breeding is still an effective way for crop improvement, modern technologies such as marker-trait associations have fast-tracked the understanding of natural variation pertaining phenotypic and genotypic diversities for further utilization in breeding programs [4–7]. Screening phenotypes with genotypic differences that can be manipulated is of fundamental interest in crop improvement. The inherited relationship between phenotypic and genotypic differences has been extensively studied in plants to understand the complex genetic architecture underlying agronomically important traits [8–10]. Phenotypic variation can be traced back to causative loci using quantitative trait loci (QTLs) and association mapping approaches [11]. Genome-wide association studies (GWAS) enable us to overcome some limitations of the QTL mapping approach and provide a relatively high resolution to comprehend allelic diversity [12]. GWAS have been extensively employed to identify the causative variations underlying specific traits in many crops, such as wheat [13, 14], rice [15, 16], cotton [17, 18], maize [19, 20], potato [21, 22] and yams [23–26]. For instance, Gatarira et al. [27] identified several putative candidate genes associated with dry matter accumulation and oxidative browning in *Dioscorea alata* using GWAS. Cormier et al. [28] conducted GWAS to decipher the genetic regulators of flowering control and sex determination in greater yam. Recently, Dossa et al. [29] located the genomic regions controlling tuber flesh color, oxidative browning and culinary traits in *D. alata*.

Yams (*Dioscorea* spp.) are considered major food security crops in Africa and other regions in the world [30, 31]. *Dioscorea rotundata*, *D. cayenensis*, and *D. alata* are the most important yam species in West and Central Africa [32]. *Dioscorea alata* L., commonly known as greater yam or water yam, is widely distributed worldwide and significantly contributes to food security [27, 33]. In West Africa, greater yam is mainly cultivated in three ecological zones, including rainforests, the southern Guinea savanna, and the wetter portion of the northern Guinea savanna [34]. Rainfalls are the primary source of irrigation in yam-cultivated areas in Africa and 1000 to 1500 mm rainfall during the cropping season favors its production [34]. Leaf development, tuber initiation, and bulking are the critical growth stages, and a lack of sufficient water can reduce the yield to critical levels [35]. Diby et al. [36] reported a significant yield decrease in *D. alata* under water stress conditions. Moisture stress can

directly impact dry matter accumulation due to low photosynthetic rate and metabolism, indirectly impacting tuber initiation and development [37, 38]. There is a consensus on developing drought and heat-tolerant greater yam cultivars for stable and improved tuber yield performance [37]. Climate change, unpredicted rainfalls, and increasing temperatures constantly threaten yam production [39, 40]. Moreover, the lack of studies concerning abiotic stress tolerance in yam requires attention to cope with the emerging challenges associated with climate change and food security [36].

Stress tolerance is a multi-stage phenomenon comprising stress sensing, signaling, and recovery [41]. Understanding the mechanisms underlying plant stress responses is crucial for improving crop resilience to abiotic and biotic stresses. Traits such as leaf dry matter content (LDMC), mean leaf area, net photosynthesis rate, transpiration rate, transpiration use efficiency (TUE), stomatal density, stomatal index, and leaf thickness are vital for stress adaptation. High LDMC, for instance, is associated with drought-resistance, while smaller leaf areas reduce transpiration and heat load under arid conditions [42, 43]. Net photosynthesis is critical for energy production, but its efficiency can decline under water-limited or saline conditions [44]. Traits like high TUE enhance biomass production per unit of water used, an essential adaptation for drought tolerance [45]. Meanwhile, stomatal density and index regulate gas exchange and water loss, balancing carbon fixation with water conservation [46]. Ecological strategies such as competitor, stress-tolerator, and ruderal (CSR) also provide insights into resource allocation and survival strategies across varying environmental conditions [47]. These traits and strategies collectively interact to mitigate stresses such as drought, salinity, extreme temperatures, nutrient deficiencies, herbivory, and pathogen attacks. While these relationships have been extensively studied in crops like rice, corn, wheat, soybean, and cowpea, they remain underexplored in yams.

Research to unravel the genetic basis of major agronomic traits associated with stress response will have far-reaching implications for the genetic improvement of *D. alata*. This is the first such study aimed at deciphering the genetic architectures of leaf morpho-physiological attributes related with stress response in *D. alata*. To achieve this objective, a diversified panel of *D. alata* was assembled and phenotyped for the studied traits at two locations. Thanks to the reference genome of *D. alata* v2.1 [48], we conducted GWAS to detect the genomic variations and putative candidate genes associated with the studied traits. The results provide a framework for

further improvement in breeding programs concerning the development of climate-resilient *D. alata* cultivars.

Results

Leaf morpho-physiological characterization

Summary statistics showed significant variation for the 12 traits in the association panel, which is useful for deciphering their genetic architectures (Table 1 and Fig. S1). The broad-sense heritability (H^2) computed for the different traits ranged from 0.48 stress-tolerator ecological strategy (S) to 0.94 transpiration rate (E).

Furthermore, comparisons of these traits at the two planting locations suggested the planting sites significantly affected transpiration use efficiency (TUE), E, leaf thickness (LT), S, and ruderal ecological strategy (R) variations within the panel (Fig. 1). Pearson's correlation between traits was estimated, where negative and strong correlations were observed between E and TUE; S and R (Fig. 2A). Mean leaf area (LA1) was positively correlated with competitor ecological strategy (C), while negatively correlated with R (Fig. 2A). Interestingly, we observed weak correlations between most of traits, suggesting that breeding simultaneously for some of these stress responses-related traits would be possible.

Figure 2B represents the ecological strategy of yam genotypes as classified by Grime [49] into competitive ability (C), physiological tolerance to stress (S) and ruderality, i.e., adaptation to disturbance (R). There is quite a large diversity of physiological tolerance to stress (S-R axes) and the genotypes exhibited an important phenotypic plasticity illustrated by different behaviors in the two environments with contrasting climatic conditions (Fig. 2B and Fig. S2).

Genotyping and genome-wide association studies

A total of 1.9 million high-quality SNPs were used for genome-wide association study (GWAS). The advanced multiple loci statistical model “Bayesian-information and linkage-disequilibrium iteratively nested keyway” implemented in the R package GAPIT3 [50] was employed. It has been proven more robust than the traditional Mixed Linear Model [51]. In this study, GWAS was independently conducted using phenotypic data from each location to identify environment-specific QTLs as well as stable QTLs across locations. The threshold for significant associations was set at $\log_{10} P=6$.

At the two locations, we identified significant associations for all the traits, except for ruderal ecological strategy. We observed that for traits with high H^2 , similar genomic regions were stably identified at the two locations. For example, for stomatal density, which has an $H^2=0.87$, a strong SNP rs1744535 at position 22,836,023 on Chromosome (Chr) 18 was identified at Godet (Fig. 3A, B and Table 2) while a close SNP rs1737071 at position 22,259,955 on Chr18 was detected at Duclos (Fig. 3C, D). Likewise, for transpiration rate, which has a very high $H^2=0.94$ in our panel, GWAS resulted in a significant SNP rs205735 at position 326,438 on the Chr3 at Duclos and a close SNP rs202363 at position 346,038 on Chr3 at Godet (Fig. 3E-H).

However, this is not true for traits with lower H^2 . For instance, for leaf dry matter content we identified significant SNPs from different genomic regions (Chr5 and Chr13) at Godet and Duclos (Fig. 4A-D). Similarly, two SNPs on Chr15 (rs1436584) and Chr3 (rs264342) were identified for stomatal index at Godet and Duclos, respectively (Fig. 4E-G). Overall, 24 significant

Table 1 Summary statistics for leaf morpho-physiological traits associated with stress response in greater yam

Traits	Unit	Min	Max	Average	SE	V	SD	CV	G	GxE	H ²
LDMC	%	0.10	0.27	0.18	2,25E-03	0.00136	369.00	20.43	***	*	0.58
LA1	cm ²	7.32	85.67	32.37	1.04	288.21	16.98	52.44	***	ns	0.92
A	μmolCO ₂ M ⁻² s ⁻¹	0.95	23.33	12.65	0.20	19.63	4.43	35.04	***	ns	0.81
E	mol m ⁻² s ⁻¹	0.48	4.45	2.66	374.00	0.60	0.77	29.00	***	***	0.94
TUE	μmolCO ₂ mmol ⁻¹ H ₂ O	0.38	9.15	4.83	0.06	1.81	1.34	27.82	***	***	0.76
IS	%	8.89	37.14	22.74	0.22	23.22	4.82	21.19	***	ns	0.77
DS	mm ⁻²	50.44	353.10	170.69	2.62	3366.38	58.02	33.99	***	ns	0.87
NN	NA	3.00	15.00	9.42	0.15	6.07	2.46	26.16	***	ns	0.74
LT	mm	0.2000	0.6000	0.3600	0.0048	0.0058	0.0759	20.80	***	**	0.72
S	%	0	64.95	24.54	1.65	137.01	11.46	47.02	***	**	0.48
C	%	35.05	71.6	51.90	0.94	42.42	6.49	12.49	***	**	0.77
R	%	0	45.9	23.56	1.49	108.73	10.32	44.09	***	**	0.49

LDMC Leaf dry matter content, LA1 Mean leaf area, A Net photosynthesis, E Transpiration rate, TUE Transpiration use efficiency, IS Stomatal index, DS Stomatal density, NN Preformed node count, LT Leaf thickness, C Competitor, S Stress-tolerator, R Ruderal. Nb Number of values, Min Minimum, Max Maximum, SE Standard error, V Variance, SD Standard deviation, CV Coefficient of variation, H² Broad sense heritability, G Genotype, GxE Genotype-Environment interaction, ns Non-significant difference

* significant difference at $P<0.05$
** significant difference at $P<0.01$
*** significant difference at $P<0.001$

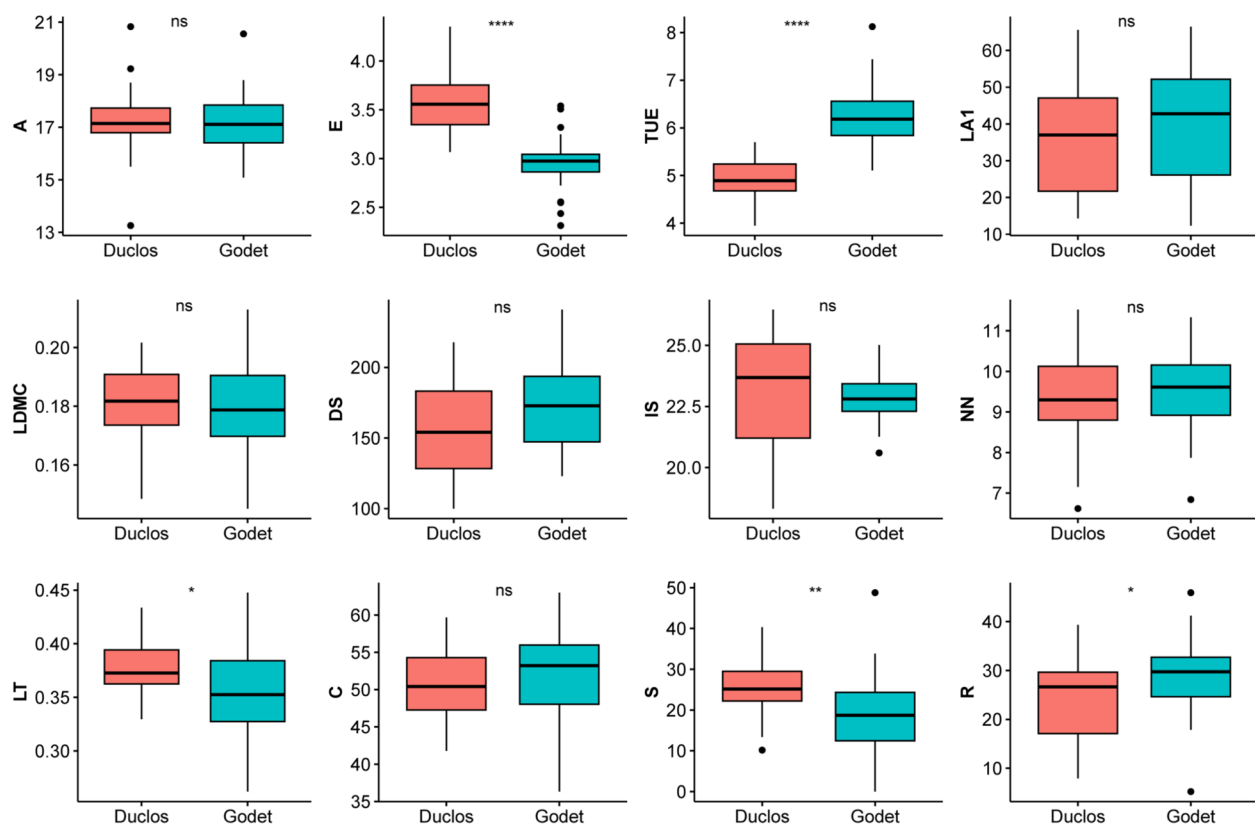


Fig. 1 Leaf morpho-physiological trait characterization at two planting sites, Duclos and Godet. Where LDMC=leaf dry matter content, LA1 = mean leaf area, A=net photosynthesis, E=transpiration rate, TUE=transpiration use efficiency, IS=stomatal index, DS=stomatal density, NN=preformed node count, LT=leaf thickness, C=competitor, S=stress-tolerator, R= ruderal ecological strategies. * significant difference at $P < 0.05$, ** significant difference at $P < 0.01$, ***significant difference at $P < 0.0001$, and ns refers to statistically non-significant difference

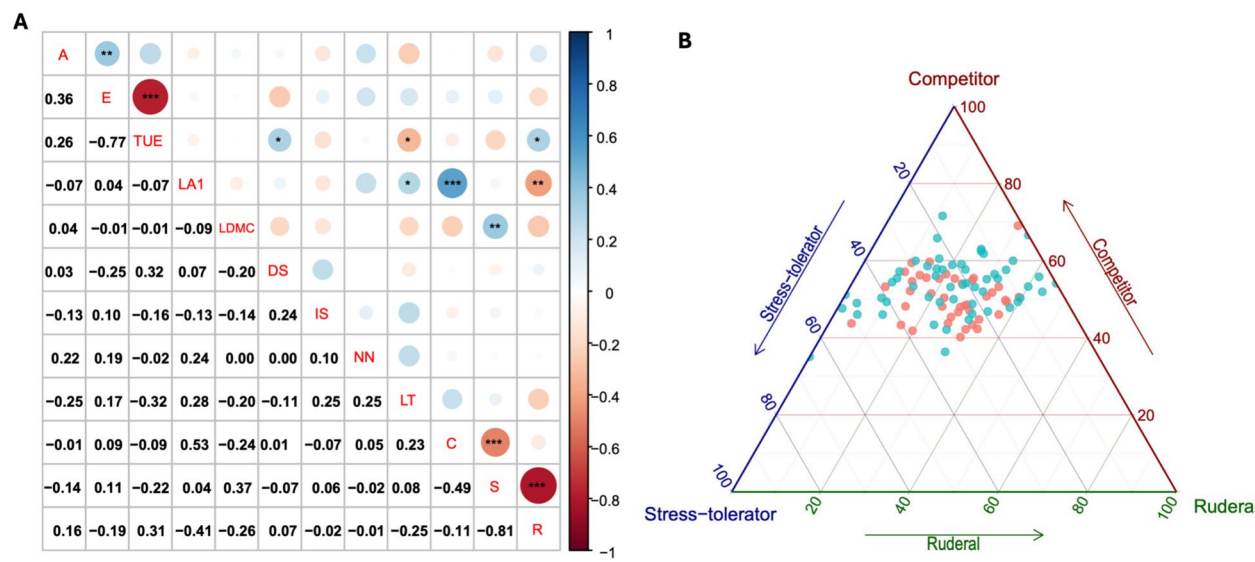


Fig. 2 Correlation among traits and overview of the competitor, stress-tolerator, ruderal (CSR) ecological strategies in our diversity panel. **A** Pearson's correlation estimates among studied traits; **B** Position of yam genotypes of the *D. alata* diversity panel on Grime's CSR triangle. Turquoise dots correspond to dryer site (Godet) and Salmon dots from wetter site (Duclos); Where LDMC=leaf dry matter content, LA1 = mean leaf area, A=net photosynthesis, E=transpiration rate, TUE=transpiration use efficiency, IS=stomatal index, DS=stomatal density, NN=preformed node count, LT=leaf thickness, C=competitor, S=stress-tolerator, R= ruderal ecological strategies; * significant difference at $P < 0.05$, ** significant difference at $P < 0.01$, *** significant difference at $P < 0.001$

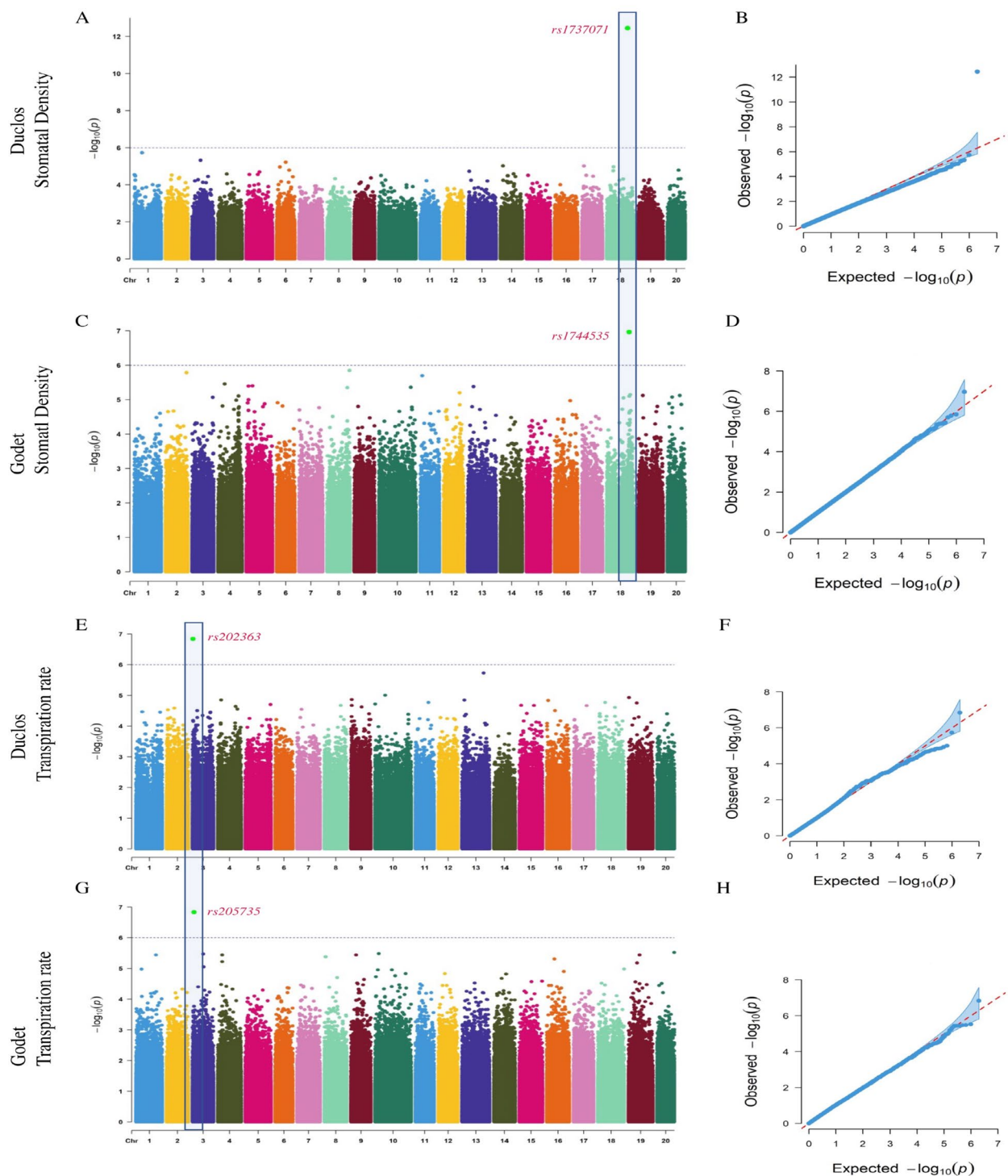


Fig. 3 GWAS for stomatal density (DS) and transpiration rate (E) in *D. alata*. **A** Manhattan plot for DS at Duclos, with the peaks indicating significant GWAS signals and the dotted horizontal lines indicating the genome-wide significance threshold, **(B)** The QQ-Plot associated with DS at Duclos shows the $-\log_{10}P$ of the expected vs. observed P values of each SNP (blue dots). The red line is a guide for the perfect fit to $-\log_{10}P$. The shaded area shows the 95% confidence interval for the QQ-plot under the null hypothesis of no association between the SNP and the trait, **(C)** Manhattan plot for DS at Godet, **(D)** The QQ-plot associated with the DS at Godet. **E** Manhattan plot for transpiration rate at Duclos, with the peaks indicating significant GWAS signals, and the dotted horizontal lines indicating the genome-wide significance threshold, **(F)** The QQ-Plot associated with transpiration rate at Duclos shows the $-\log_{10}P$ of the expected vs. observed P values of each SNP (blue dots). **G** Manhattan plot for transpiration rate at Godet, **(H)** The QQ-plot associated with the transpiration rate at Godet

Table 2 Significant SNPs and their effect on stress response-related leaf traits in *D. alata*

Location	Traits	SNP	Chr	Position	Allele	p-value (-log ₁₀)	Effect (R ²)
Godet	LMDC	rs1185429	Chr13	7,719,492	G/ A	6.18E+00	9.39%
Godet	IS	rs264342	Chr3	12,192,098	T/ C	6.07E+00	15.89%
Godet	E	rs205735	Chr3	1,346,038	T/ C	6.83E+00	25.28%
Godet	DS	rs1744535	Chr18	22,836,023	A/ G	6.96E+00	38.36%
Godet	A	rs1298027	Chr14	6,889,304	C/ T	6.10E+00	23.84%
Godet	C	rs35673	Chr1	9,542,330	G /A	7.47E+00	31.44%
		rs1938821	Chr20	11,950,212	T /C	6.52E+00	10.82%
		rs1570714	Chr17	1,304,546	A /G	6.34E+00	10.49%
		rs214171	Chr3	2,944,668	C /T	6.00E+00	12.85%
		rs304565	Chr3	19,525,040	A /G	6.14E+00	12.11%
		rs185442	Chr2	18,993,473	G /A	6.11E+00	19.56%
		rs1894629	Chr20	1,613,539	G/ A	6.06E+00	9.60%
		rs1587198	Chr17	4,889,485	G/ A	6.40E+00	19.89%
		rs1254341	Chr13	24,382,368	T /C	6.26E+00	16.32%
		rs1414555	Chr15	13,757,209	T /C	6.12E+00	14.68%
Duclos	NN	rs1330927	Chr14	14,848,380	A/ T	6.07E+00	10.05%
		rs1091838	Chr12	5,760,157	G/ A	1.21E+01	31.32%
		rs450349	Chr5	9,839,496	G/ A	6.25E+00	11.36%
		rs401519	Chr5	80,613	C /T	7.19E+00	22.84%
		rs1436584	Chr15	19,347,003	T/ A	6.20E+00	15.80%
		rs202363	Chr3	326,438	G /A	6.84E+00	9.23%
		rs1737071	Chr18	21,259,955	G/ A	1.24E+01	36.09%
		rs1610426	Chr17	9,692,719	A/ T	6.28E+00	22.19%
		rs1111390	Chr12	10,073,121	T/ G	6.13E+00	24.42%

LMDC Leaf dry matter content, LA1 Mean leaf area, A Net photosynthesis, E Transpiration rate, TUE Transpiration use efficiency, IS Stomatal index, DS Stomatal density, NN Preformed node count, LT Leaf thickness, C Competitor, S Stress-tolerator ecological strategies

The alleles in bold letters correspond to the favorable alleles

marker-trait associations were detected in this study (Fig. S3 and Table 2).

Characterization of peak SNPs for their allelic effects on the traits

The favorable alleles at each significant locus were identified and the phenotypic variance explained was computed (Table 2). For example, the SNP (Chr18: rs1737071) could explain 36% of the stomatal density variation in our panel at Duclos (Table 2). We observed that the heterozygote accessions (GA) at this locus had significantly higher stomatal density than the homozygous accessions (GG) (Fig. 5). Hence the accessions with the GA genotype could better control water loss rate and CO₂ uptake [52]. Similarly, SNP (Chr5: rs450349) explained 11.36% of the variation with the allele GA associated with higher leaf dry matter content compared to GG (Fig. 5). High stomatal density and leaf dry matter content have been linked to improved photosynthetic induction and biomass production in plants [53]. Moreover, other peak SNPs detected at Duclos, and the nine peak SNPs detected at Godet were characterized for their favorable alleles (Figs. 5 and 6).

Identification of candidate genes associated to the stress response leaf traits

We searched for candidate genes controlling the studied traits around the associated significant SNPs in a window size of 5 kb [7]. Overall, 44 genes with diverse biological functions were extracted. Sixteen genes with known functions were identified as putative candidates for physiological attributes associated with stress response. For the stomatal density trait at Duclos, the peak SNP was located between two genes (*Dioal.18G049200* and *Dioal.18G049300*) (Table 3). There was no functional annotation for *Dioal.18G049200*, but the gene *Dioal.18G049300* was annotated as a 3-keto acyl-coenzyme A synthase. At Godet, the peak SNPs were identified downstream of *Dioal.18G055600* annotating *Glutathione S-transferase*. We speculate that *Dioal.18G049300* and *Dioal.18G055600* could be the potential regulators of stomatal density in *D. alata*.

Net photosynthesis showed one association signal rs1298027 on Chr14, accounting for 23.84% of the phenotypic variance (Table 2). The peak SNP was located between *Dioal.12G033500* and *Dioal.12G033600*, annotated as *ABC-2 type transporter family protein* and *Phosphatidyl inositol monophosphate 5 kinase 4*, respectively. Moreover, three putative candidate

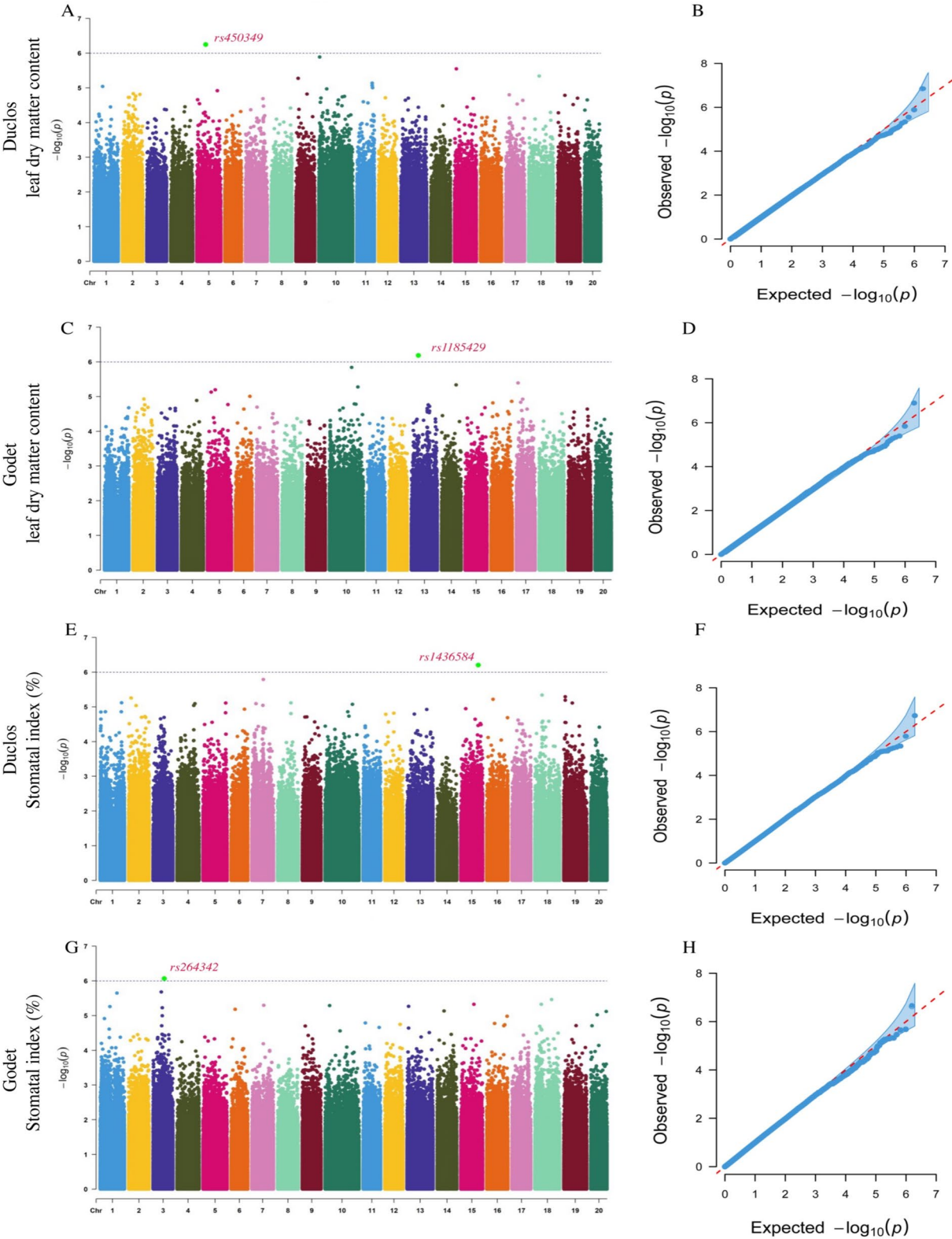


Fig. 4 (See legend on next page.)

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Fig. 4 GWAS for leaf dry matter content and stomatal index in *D. alata*. **A** Manhattan plot for leaf dry matter content at Duclos, with the peaks indicating significant GWAS signals, and the dotted horizontal lines indicating the genome-wide significance threshold, **(B)** The QQ-Plot associated with leaf dry matter content at Duclos shows the $-\log_{10}P$ of the expected vs. observed P values of each SNP (blue dots). The red line is a guide for the perfect fit to $-\log_{10}P$. The shaded area shows the 95% confidence interval for the QQ-plot under the null hypothesis of no association between the SNP and the trait, **(C)** Manhattan plot for leaf dry matter content at Godet, **(D)** The QQ-plot associated with the leaf dry matter content at Godet. **(E)** Manhattan plot for stomatal index at Duclos, with the peaks indicating significant GWAS signals, and the dotted horizontal lines indicating the genome-wide significance threshold, **(F)** The QQ-Plot associated with stomatal index at Duclos shows the $-\log_{10}P$ of the expected vs. observed P values of each SNP (blue dots). **G** the Manhattan plot for stomatal index at Godet, and **(H)** The QQ plot associated with the stomatal index at Godet

genes (*Dioal.03G019500*, *Dioal.03G019600*, and *Dioal.03G004300*) for transpiration rate, five (*Dioal.03G073900*, *Dioal.03G074000*, *Dioal.02G062100*, *Dioal.20G005200*, and *Dioal.20G005300*) for transpiration efficiency, and four (*Dioal.03G049700*, *Dioal.03G049800*, *Dioal.15G069300*, and *Dioal.15G069400*) for stomatal index were identified, some containing exonic and intronic SNPs.

For leaf morphological traits and preformed node count, 13 putative candidate genes were identified on different chromosomes. *Retrotransposon protein*, *Neprosin_AP*, and *Chloroplast sensor kinase* were identified as putative candidate genes for leaf dry matter content. *Dioal.17G048200*, *Dioal.17G048300*, *Dioal.13G076500*, *Dioal.15G047700*, *Dioal.15G047800*, *Dioal.14G073400*, and *Dioal.14G073500* were identified for preformed node count (Table 3). *Domain of unknown function* (*DUF4228*), *Non-specific serine/threonine protein kinase*, and *DNA/RNA helicase protein* were identified as candidate genes for mean leaf area and leaf thickness.

For competitor and stress-tolerator ecological strategies, we identified 11 genes around the significant SNPs with some genes (*Iron/ascorbate oxidoreductase*, *F-box domain* and *S-adenosylmethionine*) well known to be involved in stress responses in plants (Table 3) [54–57].

Gene ontology enrichment analysis of the candidate genes highlighted several terms related to leaf morphogenesis and stress response, including regulation of gene expression, regulation of photosynthesis, wax biosynthetic process, response to water deprivation, cuticle development, stomatal movement, cellular response to starvation, auxin-activated signaling pathway (Fig. 7). All these results suggest that the candidate genes detected in this study have potential for enhancing stress response in *D. alata* genotypes.

Discussion

In this study, we investigated the genetic basis for several leaf traits related to stress response through genome-wide association studies (GWAS). Although QTL mapping and GWAS have been successfully implemented for various traits in yams [23, 25–28, 58, 59], stress-related traits have not yet been characterized using GWAS or other genetic approaches [7].

In this study, GWAS resulted in the identification of 24 significant associations with a stringent threshold

($\log_{10}P=6$), which was much higher than previously published reports [23, 25, 28]. A stringent threshold increased the significance of identified SNPs and candidate genes but may have reduced the number of significant associations [7]. Despite successfully identifying genetic variants associated with these traits, the phenotypic variation explained are much lower relative to heritability estimates. This has been reported to be related to the presence of structural variations, genotype-environment interactions, epistasis, origin, and errors in heritability estimates [60, 61]. However, we identified consistent associations at two locations where trait heritability estimates were high (E and DS). These results are consistent with previous reports suggesting the identification of stable QTLs for traits with high heritability [62, 63].

Identification and characterization of alleles associated with key SNPs enable us to understand the effect of significant associations on the leaf traits [7, 64]. Response to abiotic stress is a complex mechanism [65]. During stress, photosynthesis is mainly affected, and plants tend to close their stomata, simultaneously restricting the inflow of CO_2 and reducing the photosynthesis rate [66]. We identified 24 advantageous alleles with positive contributions to the studied traits. It is important to mention that although the traits studied are known to be involved in stress response in plants, further studies are needed to check how the favorable alleles at the marker-trait associations influence stress response in greater yam genotypes. Hence, abiotic stress experiments can be carried out using selected genotypes in our diversity panel to confirm the relationship between the leaf traits, the alleles at the associated SNPs and stress response. Genotypes with advantageous alleles (associated with trait performance) could be further utilized in breeding programs to accumulate the desirable variation for developing resilient cultivars [67, 68]. Genomic-assisted breeding which has been successful in the development of resilient crops against stress, such as wheat [69, 70] and rice [71, 72] could be applied in greater yam.

The gene *Dioal.18G049300* annotated as 3-keto acyl-coenzyme A synthase (*KCS*) enzyme was identified as a putative candidate gene for DS, which is involved in synthesizing very long-chain fatty acids. It has been demonstrated that the *Arabidopsis thaliana* *KCS* gene (*hic*) controls stomatal development through CO_2 perception,

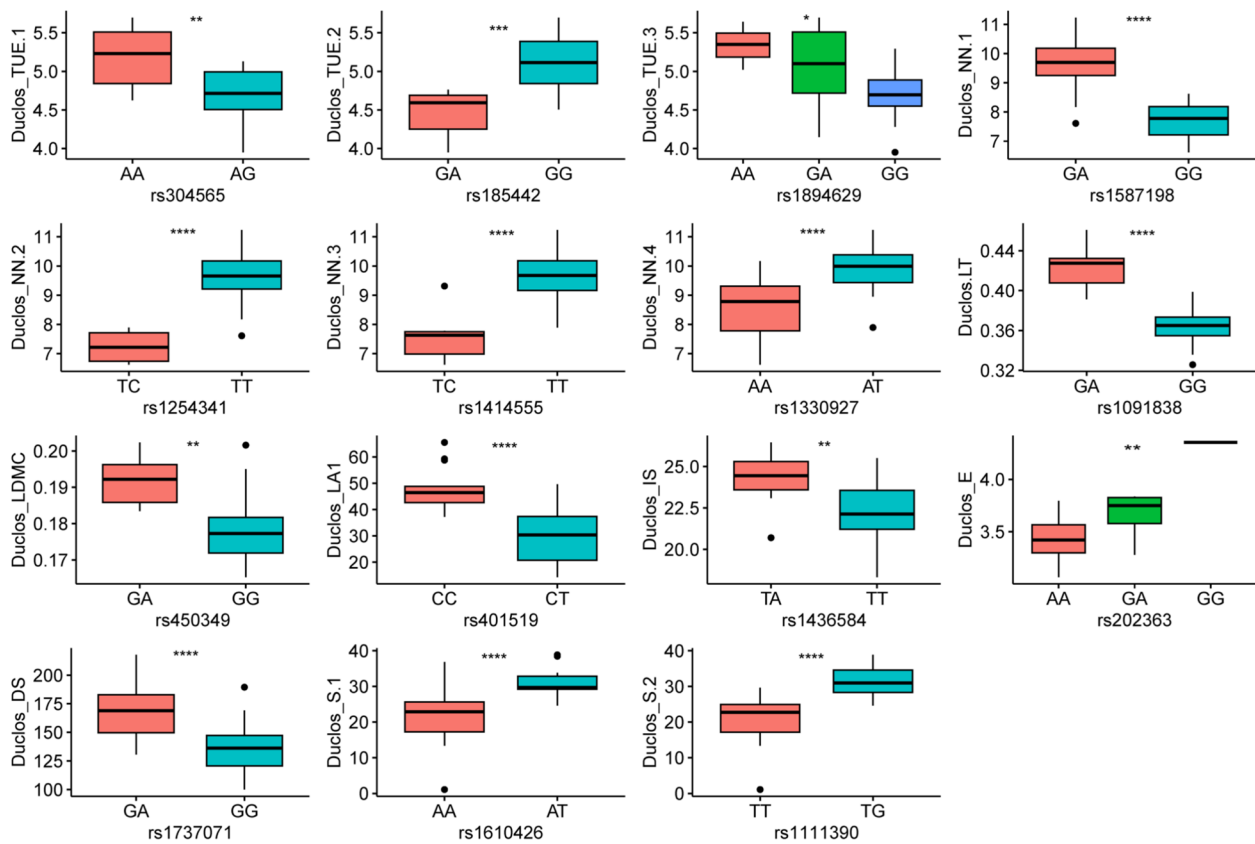


Fig. 5 Allelic effect of 15 significant SNPs identified at Duclos. LDMC=leaf dry matter content, LA1 = mean leaf area, A = net photosynthesis, E = transpiration rate, TUE = transpiration use efficiency, IS = stomatal index, DS = stomatal density, NN = preformed node count, LT = leaf thickness, S = stress-tolerator ecological strategy. * significant difference at $P < 0.05$, ** significant difference at $P < 0.01$, *** significant difference at $P < 0.001$ and **** significant difference at $P < 0.0001$

and the mutant plants displayed a 42% increase in stomatal density in response to elevated CO_2 [73]. Another gene *Dioal.18G055600* was identified, annotated as *Glutathione s-transferase (GST)*. *GST* has been shown to control plant response to a wide range of biotic and abiotic stress conditions [74–76].

Similarly, *Dioal.12G033500* (*ABC-2 type transporter family protein*) was identified as a putative candidate gene for net photosynthesis. A previous report suggests that disruption of *ATP-binding cassette transporters* causes deregulation of stomatal opening and increases drought susceptibility [77]; therefore, variation in *Dioal.12G033500* function or expression could explain the photosynthesis variation in the GWAS panel and could be an excellent candidate for further functional verification in *D. alata*. *Retrotransposon protein* [78], *Domain of unknown function (DUF4228)* [79, 80], *Non-specific serine/threonine protein kinase* [81, 82], *DNA/RNA helicase protein* [83, 84] have also been characterized for their potential role in coping with stress conditions and maintaining the plant growth. Gene expression data facilitate the identification of candidate genes from GWAS projects since genetic variants falling in genic or

intergenic regions often lead to non-functional genes or altered gene expression. There is no transcriptome data related abiotic stress response available in greater yam. Therefore, we recommend that a future study can generate gene expression data under various stresses to support the ongoing genetic studies.

Conclusions

We successfully employed GWAS to detect genomic signals (24) and candidate genes (44) linked to leaf traits related to stress response in *Dioscorea alata*. Traits with high heritability estimates, stomatal density, and transpiration rate, were identified with consistent GWAS signals on Chr18 and Chr3. Nonetheless, additional efforts are needed to enlarge the diversity panel to improve the GWAS power and detect more marker-trait associations. Accessions accumulating favorable alleles at the different significant loci will be used as genitors in our breeding programs. Moreover, developing allele-specific markers for the strong and stably detected significant marker-trait associations will improve marker-assisted selection, introgression, and the pyramiding of favorable alleles/genes in greater yam cultivars.

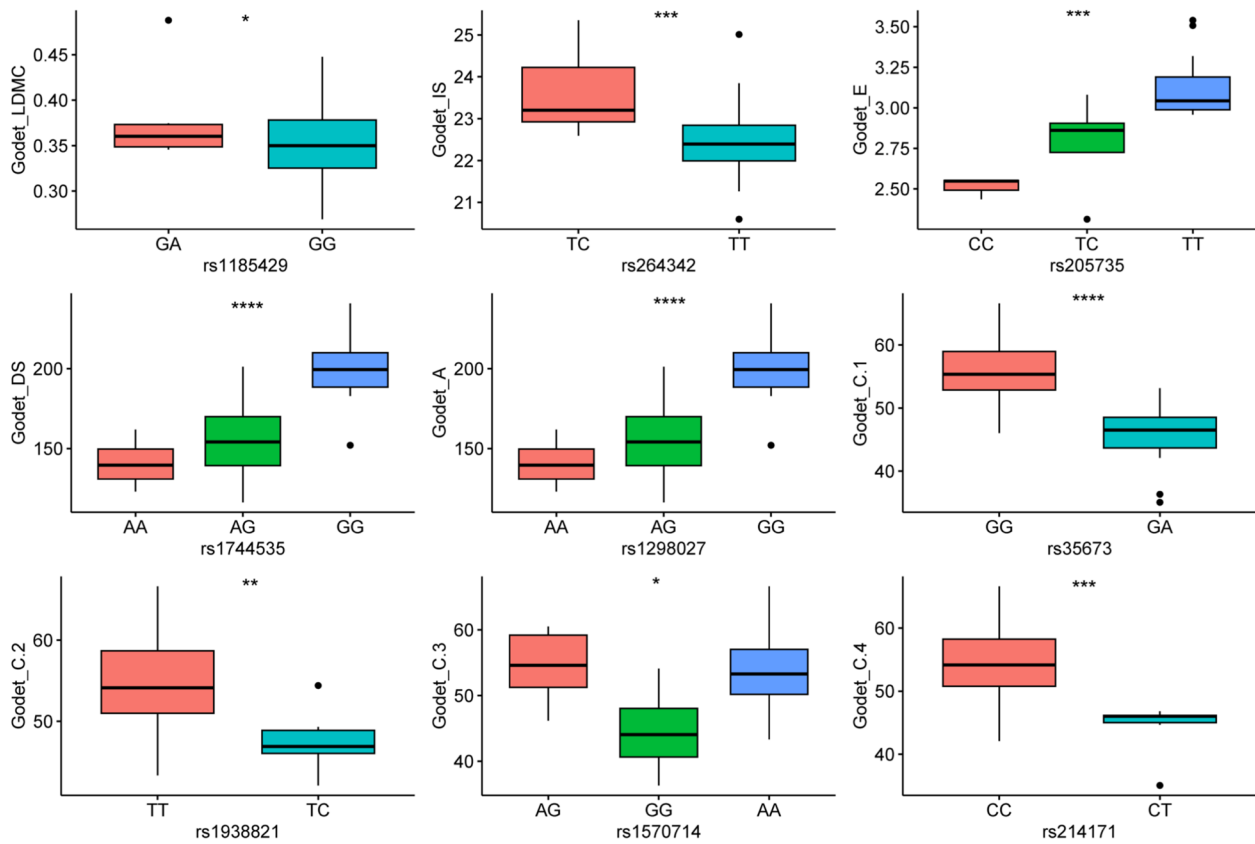


Fig. 6 Allelic effect of nine significant SNPs identified at Godet. A = net photosynthesis, DS = stomatal density, E = transpiration rate, IS = stomatal index, LDMC = leaf dry matter content, C = competitor ecological strategy. * significant difference at $P < 0.05$, ** significant difference at $P < 0.01$, *** significant difference at $P < 0.001$ and **** significant difference at $P < 0.0001$

Methods

Plant material and leaf morpho-physiological characterization

Plant materials used in this study include 53 genotypes of *Dioscorea alata* L. [28] (Table S1). This panel comprises genotypes from nine countries of the yam belt countries of west Africa, the Caribbean, and the Pacific islands. The plant materials were obtained from the germplasm collection of the Centre de coopération Internationale en Recherche Agronomique pour le Développement, Guadeloupe and the Biological Resource Centre for tropical plants in the West Indies. The plant materials were planted at two locations, Duclos (16°120' N, 61°39' O, 125 m above sea level (a.s.l.)), and Godet (16°20' N, 61°30' 0.10 m a.s.l.), in Guadeloupe. The two locations have contrasting climatic conditions (Fig. S2). A total of 10 seedlings of each cultivar were planted in three replicates (30 seedlings per genotype in total). These plants were spread over three ridges (65 m long) spaced 30 cm apart within the ridges. Cane straw was used as mulch over the entire plot to limit weeds at Godet, while paper mulch was used at Duclos. The plot was drip irrigated, and planting started in March 2021. Genotypes were planted from harvested seeds at year n-1 when 50% of the tubers

of the genotype germinated in the storage shed. All the genotypes were characterized for 12 morpho-physiological traits associated with stress response, including leaf dry matter content (LDMC), mean leaf area (LA1), net photosynthesis (A), transpiration rate (E), transpiration efficiency (TUE), stomatal index (IS), stomatal density (DS), preformed node count (NN), leaf thickness (LT), C = competitor, S = stress-tolerator, R = ruderal ecological strategies as classified by Grime.

Traits were characterized prior to tuber initiation, between 45 and 60 days after emergence, to ensure the presence of enough mature leaves. LDMC was measured using three fully mature leaves collected from each genotype. Fresh leaf samples were always taken in the early morning hours, between 6 and 8 am, when relative humidity was still high (70–95%) and temperatures were around 25 °C. The leaves were wrapped in similarly-sized wet papers to avoid drying and transported to the laboratory in a cooler. Fresh weight was measured immediately after collection, while dry weight was determined after drying the leaves in an oven at 60 °C for three days. LDMC was calculated using the formula.

Table 3 List of candidate genes associated with stress response-related leaf traits in greater yam

Traits	SNP	Upstream the gene/ downstream the gene	Localization	Function
Godet_LMDC	rs1185429	<i>Dioal.13G049900</i> <i>Dioal.13G050000</i>	intergenic	Retrotransposon protein Neprosin_AP
Godet_IS	rs264342	<i>Dioal.03G049700</i> <i>Dioal.03G049800</i>	intergenic	SHR-BD Major facilitator superfamily protein
Godet_E	rs205735	<i>Dioal.03G019500</i> <i>Dioal.03G019600</i>	intergenic	Fatty acid hydroxylase superfamily Nodulin MtN21/EamA-like transporter family protein
Godet_DS	rs1744535	<i>Dioal.18G055600</i>	downstream	GLUTATHIONE S-TRANSFERASE
Godet_A	rs1298027	<i>Dioal.12G033500</i> <i>Dioal.12G033600</i>	intergenic	ABC-2 type transporter family protein Phosphatidylinositol monophosphate 5 kinase 4
Godet_C	rs35673	<i>Dioal.01G024000</i> <i>Dioal.01G024100</i>	intergenic	IRON/ASCORBATE OXIDOREDUCTASE RNA-directed DNA polymerase
	rs1938821	<i>Dioal.20G032000</i> <i>Dioal.20G032100</i>	intergenic	NA PROTEASE DO-LIKE 1
	rs1570714	<i>Dioal.17G018200</i>	upstream	S-ADENOSYLMETHIONINE CARRIER 2
	rs214171	<i>Dioal.03G033700</i> <i>Dioal.03G033800</i>	intergenic	Leucine Rich Repeat F-box domain and LRR containing protein
Duclos_TUE	rs304565	<i>Dioal.03G073900</i> <i>Dioal.03G074000</i>	intergenic	Ankyrin repeat family protein Seed imbibition 2
	rs185442	<i>Dioal.02G062100</i>	exonic	Non-specific serine/threonine protein kinase
	rs1894629	<i>Dioal.20G005200</i> <i>Dioal.20G005300</i>	intergenic	Protein phosphatase 2C family protein Non-specific serine/threonine protein kinase
Duclos_NN	rs1587198	<i>Dioal.17G048200</i> <i>Dioal.17G048300</i>	intergenic	CCT motif family protein UDP-D-glucose/UDP-D-galactose 4-epimerase 5
	rs1254341	<i>Dioal.13G076500</i> <i>Dioal.13G076600</i>	intergenic	Exostosin family NA
	rs1414555	<i>Dioal.15G047700</i> <i>Dioal.15G047800</i>	intergenic	Lipid transfer protein 12 Non-specific serine/threonine protein kinase
	rs1330927	<i>Dioal.14G073400</i> <i>Dioal.14G073500</i>	intergenic	S-adenosyl-L-methionine-dependent methyltransferase protein pentatricopeptide
Duclos_LT	rs1091838	<i>Dioal.12G033000</i> <i>Dioal.12G033100</i>	intergenic	NA Domain of unknown function (DUF4228)
Duclos_LDMC	rs450349	<i>Dioal.05G072500</i> <i>Dioal.05G072600</i>	intergenic	Chloroplast sensor kinase NA
Duclos_LA1	rs401519	<i>Dioal.05G000800</i> <i>Dioal.05G000900</i>	intergenic	Non-specific serine/threonine protein kinase DNA/RNA helicase protein
Duclos_IS	rs1436584	<i>Dioal.15G069300</i> <i>Dioal.15G069400</i>	intergenic	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein ARM repeat superfamily protein
Duclos_E	rs202363	<i>Dioal.03G004300</i>	intronic	Heavy metal ATPase 4
Duclos_DS	rs1737071	<i>Dioal.18G049200</i> <i>Dioal.18G049300</i>	intergenic	NA Very-long-chain 3-ketoacyl-CoA synthase
Duclos_S	rs1610426	<i>Dioal.17G055400</i> <i>Dioal.17G055500</i>	intergenic	NA Tetratricopeptide repeat domain containing protein
	rs1111390	<i>Dioal.12G035200</i> <i>Dioal.12G035300</i>	intergenic	WD domain, G-beta repeat (WD40) Retrotransposon gag protein

LDMC Leaf dry matter content, LA1 Mean leaf area, A Net photosynthesis, E Transpiration rate, TUE Transpiration efficiency, IS Stomatal index, DS Stomatal density, NN Preformed node count, LT Leaf thickness, C Competitor ecological strategy, S Stress-tolerator ecological strategy



Fig. 7 Gene ontology enrichment for the candidate genes detected around the significant SNPs associated with stress response-related leaf traits in greater yam

$$LDMC = \frac{\text{Leaf dry weight}}{\text{leaf fresh weight}}$$

$$TUE = \frac{A}{E}$$

Leaf area estimation was performed using the LiCor 3100c planimeter (LI-COR Biosciences, Lincoln, NE, USA). Leaf thickness was measured using a digital micrometer (Mitutoyo, Kawasaki, Japan). Each leaf was gently placed between the micrometer jaws, and measurements were taken at three different points along the leaf to ensure accuracy and consistency.

The estimation of the CSR ecological strategy was conducted using the methodology developed by Pierce et al. [85]. This approach involves assessing plant traits that correspond to the competitive, stress-tolerant, and ruderal strategies in plants. NN was recorded for 3 plants of each genotype and corresponds to the number of juvenile phytomers from the stem apex to the first node carrying a mature leaf.

Determination of stomatal density and index

Samples were collected in the morning before 9 am. The stomata are present on the abaxial surface of the leaf [86]. To estimate the stomatal density, the procedure was adapted from Paul et al. [87] to prepare the slides for microscopic observations using suitable replica fluid/adhesive. The slides were then observed under a microscope with 40× magnification, and 60 images were saved for each genotype with ZEN software (Carl Zeiss microscope, GmbH, version 2.3, blue version, 2011). ZEN allows us to directly convert the number of pixels to metric units and get the scale of the captured image. We calculated DS and IS by the following formula.

$$DS = \frac{Nb \text{ stomata}}{\text{Image surface}} \times 10^6$$

$$IS = \frac{Nb \text{ stomata}}{Nb \text{ stomata} + Nb \text{ cellular}} \times 100$$

where *Nb stomata* is the number of stomata per unit area, and *Nb cellular* is the number of epidermal cells per unit area.

Gas exchange measurement

To estimate the gaseous exchange (*A*, *E*, *TUE*), we used ADC LCPro + infrared gas exchange analyzer (ADC bioscience, Hodstone UK Limited). All measurements were taken from 7 to 9 am in the morning. The parameters were set as follows: temperature 30 °C, saturation brightness is 1400 μmol photon m⁻² s⁻¹, while relative humidity was stable (80%). We estimated *A* and *E* and then calculated transpiration use efficiency by the following formula

Genome-wide association studies

Dossa et al. [29] fully detailed the DNA extraction, sequencing and data processing steps. GWAS was performed with all 1.9 million SNPs using the advanced multiple loci model statistical model Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) implemented in the R package “GAPIT3” [50]. BLINK provides an efficient platform with increased power compared to other methods, while reducing the calculation time. The Manhattan plots were also generated in R4.0.23 with the “CMplot” package [88]. SNPs having a significant association with traits were determined by the adjusted *p*-value. The threshold of $P < 10^{-8}$ (0.05/*n*, with *n*=number of SNPs) was set to report a significant association. The quantile–quantile (QQ) plots were generated by plotting the negative logarithms (−log₁₀) *P*-values relative to their expected *p*-values to fit model relevance GWAS with the null hypothesis of no association and to determine to what extent the models considered the structure of the population.

Putative candidate gene identification

To inventory potential genes near associated SNP markers for target traits, we used ANNOVAR version 2.4 to identify genes downstream and upstream of the significant SNPs based on linkage disequilibrium (LD=5 Kb) [29]. The identified candidate genes were screened for their putative functional attributes. Subsequently, we extracted the related genes and conducted a Gene Ontology (GO) enrichment analysis using the KOBAS-i tool. The significantly enriched GO terms were then visualized using the “ggplot” package from the R4.0.23 software.

Statistical analysis

The effect of alleles at significant SNPs was assessed by comparing phenotyping data for haplotype groups. A Student's *t*-test was used to compare the groups of haplotypes ($P < 0.05$) in the R4.0.23 software with the “ggpubr” and “rstatix” packages.

Using the LME4 package [89] available on R4.0.23, we considered the genotype as a random effect to obtain the variance components of all the traits: Trait ~ (1|genotype) + (1|location) + (1|location:genotype). We then calculated phenotypic variance share of genetic source as broad sense heritability (H^2) according to Falconer and Mackay [90]:

$$H^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_{g \times e} / E + \sigma^2_e / N + \sigma^2_\varepsilon}$$

where σ^2g is genotype variance, $\sigma^2g * e$ is Genotype-by-environment interaction variance, σ^2e is Environmental variance, $\sigma^2\varepsilon$ is Residual variance, E is Number of environments and N is Number of replicates per genotype in each environment.

The basic descriptive analysis of the stress response-related leaf traits was performed by statistix 8.1. Pearson's correlation analysis between different stress response-related traits was performed by R4.0.23 using the “corrplot” package [91]. The differences in two planting sites for each trait were estimated using analysis of variance according to Steel et al. [92]. AOV-function in R4.0.23 was employed to perform analysis of variance. P -value < 0.05 was regarded as significant.

Abbreviations

GWAS	Genome wide association studies
SNP	Single nucleotide polymorphism
QTL	Quantitative trait loci
LDMC	Leaf dry matter content
LA1	Mean Leaf area
A	Net photosynthesis
E	Rate of transpiration
TUE	Transpiration use efficiency
IS	Stomatal index
DS	Stomatal density
NN	Preformed node count
LT	Leaf thickness
C	Competitor ecological strategy
S	Stress-tolerator ecological strategy
R	Ruderal ecological strategy
SD	Standard deviation
CV	Coefficient of variation
H^2	Broad sense heritability
BLINK	Bayesian-information and linkage-disequilibrium iteratively nested keyway
GO	Gene ontology

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-07595-3>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.

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Authors' contributions

Conceptualization: K D, D C. Data collection: J-L I, D C, A P, B Y T. Data curation and Formal analysis: K D, M E H, D C. Funding acquisition: D C, H C. Writing ± original draft: K D. Writing ± review & editing: D C, H C. All authors have read and approved the final version of this manuscript.

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Data availability

The Illumina NovaSeq 6000 sequencing raw data are available in the NCBI Sequence Read Archive, under the BioProject number: PRJNA918625 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA918625>). The phenotypic datasets are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

All relevant institutional, national, and international guidelines and legislations were followed while conducting this experiment. The plant materials are available at the germplasm collection of the Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Guadeloupe.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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